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Congenital Aortic Stenosis

A Retrospective Study on 32 Operated Patients

M Vukas L E Carlgren and J Swedberg

From the Departments of Thoracic Surgery and Pediatric Cardiology University of Goteborg Goteborg Sweden

ABSTRACT The magnitude of the peak systolic pressure gradient (PSPG) over the aortic valve was often the determining factor when deciding for or against operation of the congenital aortic stenosis The impression that PSPG alone was an inadequate parameter for assessment of the degree of stenosis was supported by the present retrospective analysis of 32 patients operated upon for this anomaly A gradient of 75 mmHg or more indicates a tight stenosis but a lower value does not exclude a pronounced constriction of the orifice, any more than does absence of symptoms or signs of left ventricular hypertrophy (LVH) on the ECG It is probably not only the size of the stenotic orifice that determines the prognosis in aortic stenosis but also other factors, e g function of the left ventricular myocardium A hypothesis was put forward by one of us (M V) that the location of the valvular ostium, central or peripheral, can be of importance and that turbulence induced vibrations of the left ventricular wall may depress the myocardium Differences in the pathoanatomy of the stenotic aortic valves did not explain the poor correlation between e g symptoms or LVH and PSPG or the size of the remaining orifice in the present study

During preoperative investigation of patients with congenital aortic stenosis there are sometimes large discrepancies between symptoms and pathological signs on the ECG or heart X ray or at heart catheterization on the one hand and intraoperative findings on the other as well as between the different variables Several authors have previously reported unexpected pre and intraoperative deaths even in patients with relatively low peak systolic pressure gradient (PSPG) (4 5) It seems that the PSPG itself does not give sufficient information about the *prognosis in patients with congenital* aortic stenosis and is thus an inadequate criterion *of the indication for operation*

This retrospective analysis of our operations was carried out in an endeavour to find some other parameters which might provide better information about the degree of anomaly

STUDY POPULATION

The study population comprised 32 patients 24 males and 8 females with congenital aortic stenosis Most cases were investigated at the Department of Pediatric Cardiology University of Goteborg and operated upon between Jan 1st 1967 and Dec 31st 1973 at the Department of *Thoracic Surgery Their ages varied between 11 months* and 31 years 8 being above 16 years of age

Twenty eight patients had valvular stenosis (22 with three and 6 with two cusps) Two of them had hypoplastic ring Two patients had supravalvular and 2 subvalvular stenosis (one also had a hypoplastic cusp) One of the patients with valvular stenosis also had a ventricular sep*tal defect (VSD) and another had a tight pulmonary* stenosis Five patients had symptoms of heart failure during early infancy Two of them had patent ductus arteriosus which was closed at 4 and 6 months of age respectively and one had a VSD Only one of these 5 patients (11 months of age) was slightly decompensated at the time of operation Thirteen patients were completely *symptomless at the time of operation and 4 had only mild* symptoms (slightly decreased work capacity) In 15 patients moderate or severe symptoms were registered pronounced decrease in work capacity chest pain (angina pectoris) and/or syncope

METHODS

Phonocardiogram

A typical systolic murmur was registered on the PCG in all cases Elema s mingograph type 61 and an EMT 25 B microphone amplified by an EMT 22 phonopreamplifier with an inbuilt filter spectrum were used

Oakley and Hallidie Smith (3) found a correlation be*tween PSPG and the location of the maximum murmur in*

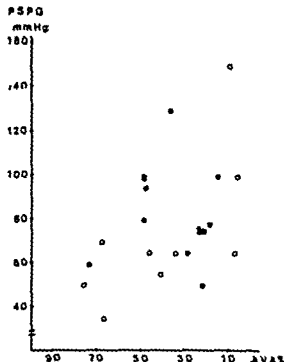


Fig. 1. Relationship between peak systolic pressure gradient (*PSPG*) and aortic valve area as percentage of the normal valve area for age (*AVA%*). (All cases with PSPG ≥75 mmHg had AVA% ≤50%. Of 12 patients with PSPG <75 mmHg, 8 had AVA% <50%. Symptomatology correlated poorly with both PSPG and AVA%.) ○=Cases with with mild symptoms, ●=cases with severe symptoms.

systole, but duration had no significance. We therefore took into consideration only the location of the maximum murmur in systole using the PCG tracing registered over the 2nd right intercostal space. Cases of combined vitium (AS+VSD, AS+PS) were excluded.

Electrocardiogram

The usual standard 12 lead ECGs were registered with Elema's mingograph type 61. Left ventricular hypertrophy (LVH) was assessed according to the method of Ahmurung et al. (1) and Ziegler (6), slightly modified.

The patients were divided into four groups: 1) No LVH. 2) Suspected or slight LVH. Amplitude of the S wave in V_1 or V_2 and/or the R wave in V_5, V_6 or aVF leads above the 90th percentile but less than the maximum normal value. 3) Moderate LVH. Amplitude of the S wave in V_1 or V_2 and/or the R wave in V_5, V_6 or aVF exceeding the normal maximum value, and/or pathologically increased ventricular activation time (>0.04 sec). 4) Severe LVH. As above but added signs of LV "strain", i.e. ST-T segment depressed by more than 1 mm and amplitude of the T wave in V_5 and V_6 less than +0.1 mV.

Heart catheterization

The LV pressures were usually registered by transseptal catheterization except in two cases in which the LV was not reached. The simultaneous pressure registrations were carried out by catheters in the LV and either in the ascending aorta, the brachial artery or in the iliac artery. In a few cases the intra-arterial pressure registration was replaced by cuff pressure measured with a sphygmomanometer in the right upper arm with the patient in the supine position. The PSPG was determined only at rest, with the patient well sedated. Synchronous registration of cardiac output was performed in only a few cases.

Indications for operation

The criteria for operation were based on the following factors, singly or together: 1) Syncopal attacks. 2) Precordial chest pain. 3) Considerably reduced work capacity. 4) PSPG over the aortic valve of at least 50 mmHg (only two patients had lower gradients). 5) Pronounced or progressive LVH.

Operative procedure

All the patients were operated upon using the heart-lung machine with a Rygg-Kyvsgaard bubble oxygenator on a Sarn console. The temperature was reduced to 28–30°C.

Twenty-seven out of 28 patients with valvular stenosis underwent open commissurotomy. In the remaining patient, who had combined aortic stenosis and insufficiency, a free fascia lata prosthesis was inserted ad modum Senning. It had to be replaced by a Björk-Shiley prosthesis 4 years later. In the two patients with discrete subvalvular

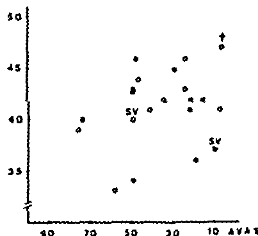


Fig. 2. Relationship between maximum murmur position in systole and aortic valve area as percentage of the normal range for age (*AVA%*). All patients with a late maximum murmur position (>40% of systolic time from 1st sound) had AVA% ≤50%, but low AVA% was also found in 3 patients with an early maximum murmur position (<40% of systolic time from 1st sound). †=Died at operation; other symbols as in Fig. 1. SV=supravalvular stenosis.

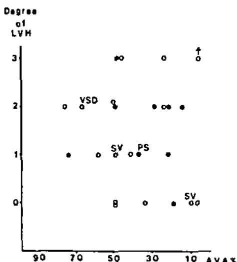


Fig 3 Relationship between the degree of left ventricular hypertrophy (*LVH*) and aortic valve area as percentage of the normal value for age (*AVA %*) Correlation was poor VSD=Ventricular septal defect PS=pulmonic stenosis Symbols and other abbreviations as in Figs 1 and 2

stenosis the membrane was excised One of these patients with a hypoplastic cusp developed aortic insufficiency after commissurotomy carried out at the same time and was later reoperated Here too a Bjork–Shiley prosthesis was inserted The waist formed supravalvular stenosis of the ascending aorta was widened with a diamond shaped teflon patch

Operative results

Out of 32 patients subjected to surgery two died A 6-year-old boy with valvular stenosis died in LV failure 10 hours after the operation The other patient who had valvular stenosis and a hypoplastic valve ring died also in LV failure during reoperation for aortic insufficiency 4½ years after the commissurotomy when a fascia lata prosthesis was inserted Both had extreme myocardial hypertrophy at necropsy All the other patients with valvular aortic stenosis operated upon by open commissurotomy had no symptoms at the 3 month control apart from slight effort dyspnoea in some cases Even at this time however two patients had clinical signs of aortic regurgitation and later another six were found to have significant clinical aortic insufficiency The patients with supra and subvalvular aortic stenosis had no symptoms at all during the entire follow up period

A postoperative diastolic murmur without clinical signs of insufficiency was registered in another 13 of the 27 patients operated upon by commissurotomy Patients with fascia lata prostheses developed advanced regurgitation but only a short diastolic murmur was observed in patients with Bjork–Shiley prostheses

Intraoperative findings

The pathological findings observed during operation were carefully recorded Special attention was paid to the number of cusps type and degree of the fused commissures thickness of the cusps fibrous excrescenses calcifications and whether the stenosed orifice was located centrally or peripherally The stenosed aortic valve area (AVA) was measured with Hegar dilators in 23 cases and calculated and presented as per cent of the normal aortic valve area (AVA%) according to the normal ranges for age (2) Three cusps of approximately the same size were found in 13 patients while in 6 cases one of the cusps was obviously hypoplastic Five patients had bicuspid valves and another two were functionally bicuspid As previously mentioned the valve ring was hypoplastic in two patients

Correlative study

In order to assess the prognostic value of different symptoms and signs we had to relate them to some standard for the degree of anomaly Apart from mortality we had no such variable Clinical symptoms such as syncope or stenocardial pain a pronounced systolic pressure gradient over the aortic valve and/or severe LVH on the ECG tracing are undoubtedly unfavourable prognostic signs But the reverse absence of subjective symptoms or ECG changes and a moderate pressure gradient need not necessarily signify a good prognosis The size of the valve opening should be a measure of the severity of anomaly In 23 cases in which the remaining opening was measured it was possible to relate it to the PSPG the position of the maximum murmur in systole and the degree of LVH registered by ECG

RESULTS

As can be seen from Figs 1–3 we did not find a good correlation between any of the variables analysed PSPG of more than 75 mmHg indicated stenosis of the aortic valve by 50% or more but a lower gradient did not exclude a pronounced aortic stenosis Moderate or severe clinical symptoms signified a tight ostium but their absence did not

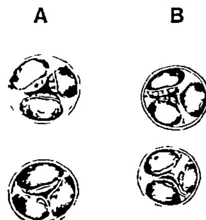


Fig 4 Sketches of typical cases with centrally (*A*) and peripherally (*B*) located stenotic orifice

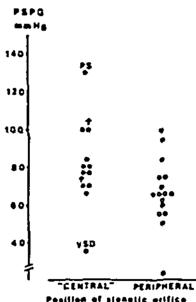


Fig 5 Relationship between peak systolic pressure gradient (*PSPG*) position of the stenotic orifice and symptoms. (Most of the patients with peripherally located orifice had PSPG <75 mmHg and most of those with centrally placed orifice had PSPG ≥75 mmHg. The difference was not statistically significant ($p<0.1$) There was no correlation between the symptoms and the different position of the orifice) Symbols and abbreviations as in Figs 1 2 and 3

lude a tight stenosis (Fig 1) Displacement of maximum of the murmur to the right in systole maximum systolic murmur >40% of systole) suggested a serious stenosis of the valve A central position or a position more to the left did not exclude this (Fig 2) The degree of LVH is poorly correlated to the size of the remaining opening (AVA%) and absence of LVH does not exclude tight stenosis (Fig. 3) No correlation was found between clinical symptoms and LVH On the recommendation of one of us (M V) all cases of valvular stenosis were divided into those with a dominantly central or peripheral location of the remaining opening (Fig 4) in order to find out whether there was any correlation between the location of the orifice and symptoms and PSPG and LVH respectively As will be seen from Fig 5 the pressure gradient tended to be lower in patients with peripheral than with central openings but the difference was not statistically significant No differences in respect of subjective symptoms were found between the groups. Nor did we find any correlation between the position of the opening and the degree of LVH (Fig. 6)

DISCUSSION

When deciding whether or not to operate great importance was attached to the magnitude of the PSPG The impression that this variable is not completely reliable is confirmed by the present study Information about cardiac output not measured in our patients is in all probability of importance for better evaluation of the significance of PSPG In this study a high PSPG certainly indicates tight stenosis but a moderately increased PSPG does not exclude pronounced constriction of the orifice any more than does absence of clinical symptoms and signs of LVH on the standard ECG Vectorcardiography was not used consistently in this retrospective study

The stenotic valve area alone is probably not a decisive factor for the occurrence of symptoms in aortic stenosis The function of the left ventricular myocardium undoubtedly also plays an important role How the myocardium is influenced is not clear Deteriorated coronary perfusion is one possible explanation Another hypothesis put forward by one of us (M V) is that turbulence induced vibrations resulting from the turbulent blood flow through the stenotic valve have an unfavourable effect on the left ventricular myocardium It is to be expected that differences in turbulence will occur in different types of stenosis and vary not only with

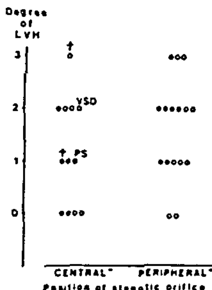


Fig 6 Relationship between left ventricular hypertrophy (*LVH*) position of stenotic orifice and symptoms. There was no correlation Symbols and abbreviations as in Figs 1 2 and 3

the size of the opening but also according to the location of the stenosed orifice centrally or peripherally A description of the pathoanatomy of the aortic stenotic valves did not add further prognostic information in the present patients The present study is however limited by the rather small number of patients and the retrospective nature of the analysis

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Embolism in Sinoatrial Disease

J Bathen S Sparr and R Rokseth

From the Section of Cardiology Regional Hospital University of Trondheim Trondheim Norway

ABSTRACT Among a series of consecutive patients treated with permanent pacemaker between 1965 and 1976 43 had sinoatrial disease with paroxysmal tachycardia (group A) 30 sinoatrial disease without tachycardia (group B) and 165 atrioventricular block (group C). A retrospective study showed systemic vascular events compatible with embolism in 35%, 7% and 10% in groups A, B and C respectively The groups were comparable as regards age other diseases and duration of pacemaker therapy Because of the high incidence of embolism in group A, anticoagulant therapy should be evaluated in patients with the brady-tachycardia syndrome

Some authors (1 4 6 7 8) have reported a high incidence of systemic embolism in sinoatrial disease while the data of others (2 3 5) suggest that the risk is negligible Because of the discrepancy there is no general agreement on prophylactic anticoagulants

The present paper shows a high incidence of thromboembolism in patients with the brady tachycardia syndrome as compared with patients with sinoatrial disease without tachycardia and patients with AV block.

PATIENTS AND METHODS

Between Sept. 1965 and Jan. 1976 245 patients have received permanent pacemaker in our clinic A retrospective study on the occurrence of thromboembolism and follow-up examination were carried out in Jan.–March 1976. The main rhythm disturbances are listed in Table I All patients had severe symptoms e g. syncope pronounced dizziness or congestive heart failure Brady cardia alternating with tachycardia was present in 43 patients (group A) sinoatrial disease without tachycardia in 30 (group B) and AV block in 165 patients (group C) Among the 73 patients with sinoatrial disease 68 had sinoatrial block or sinus arrest, while 6 had sinus bradycardia (rate less than 55) Sinoatrial block is here defined as pauses between the P waves being a multiple of the basic rate and sinus arrest as irregularly long pauses up to several seconds with a nodal escape beat at the end Patients with sinus bradycardia were included in this study only if they had symptoms which were relieved by permanent pacemaker The paroxysmal tachycardia in group A was always supraventricular (atrial fibrillation flutter and/or atrial tachycardia)

The age distribution is seen in Fig. 1 The mean age was 68 years (range 52–86) in group A 64 (range 34–85) in group B and 70 (range 41–90) in group C The sex distribution was 40% men in group A 71% in group B and 63% in group C The mean total pacing time was 39 months (range 0–108) in group A, 29 (range 10–96) in group B and 35 (range 0–107) in group C (Fig. 2)

The pacemaker electrodes were inserted via the cephalic external or internal jugular vein to the apex of the right ventricle The pulse generator was placed subcutaneously below the right or left clavicle In 3 patients with AV block the transthoracic myocardial technique was used because the transvenous technique had failed In the brady tachycardia syndrome QRS triggered or inhibited generators (Cordis Medtronic or Elema) were used except in some early patients who received fixed rate pace makers The patients were out of bed on the day after implantation, though the shoulder was immobilized for a few days. If paroxysmal tachycardia still occurred after pace maker therapy antiarrhythmic drugs mainly digitalis or *β-blockers were given.*

The patients were reexamined clinically and electrocardiographically at intervals of 3 months and more frequently after 18 months All vascular events were recorded and included in the present data. In this study the term vascular event signifies cerebral stroke with sudden transient or permanent hemiplegia or sudden peripheral vascular catastrophe with pain, pallor and loss of palpable pulsations.

RESULTS

The total mortality during observation was 33% in each group Table I shows the number of patients who died from vascular catastrophes as well as those suffering non-fatal incidents There was a

Table I. *Vascular events among 245 patients with permanent pacemaker*
A = bradycardia alternating with tachycardia. B = sinoatrial disease without tachycardia

Main reason for pacemaker therapy	No. of pats.	No. of deaths: Total	No. of deaths: From vascular events	No. of pats. with non-fatal events	Pats. with vascular events (%)
Sinoatrial disease (A)	43	14	6	9	35
Sinoatrial disease (B)	30	10	1	1	7
AV Block	165	54	7	9	10
Other reasons	7	1	0	0	0

high occurrence of systemic vascular events in group A, 35% against 7% in group B and 10% in group C.

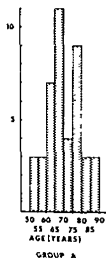
Table II shows the localization and the residual defects of the vascular incidents in group A, as well as the time of occurrence in relation to pacemaker implantation. It is noted that the incidents in 14 of 15 patients were cerebrovascular. They always occurred suddenly (within seconds or minutes) and all were compatible with embolism. Three of the patients had also pulmonary embolism, occurring in 2 of them in connection with surgery. Six patients died and 3 were seriously disabled. Two patients had transient ischemic attacks. Most vascular incidents took place after pacemaker implantation, oc-ring before (in connection with severe brady-ia) in two patients only. The average duration symptoms before pacemaker implantation was about 5 years and was not significantly different whether vascular incidents occurred or not. One patient received oral anticoagulants after suffering 2 transient ischemic attacks. Anticoagulants were discontinued before the 3rd attack occurred, then reinstituted and no further vascular incidents occurred during an observation time of 2 years. Three others received anticoagulants because of valve prosthesis. None of them had vascular incidents.

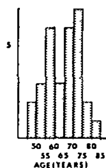
Autopsy was performed in 2 of the 6 patients who died. One autopsy showed no evidence of other diseases. A large cerebral infarction and a thrombus in the medial cerebral artery were found. The other autopsy showed that the patient had suffered an acute myocardial infarction 10 months previously. He had persistent sinoatrial disorder from the start of infarction. Extensive old myocardial infarction as well as thrombi in the right atrium and on the mitral valve were found in addition to cerebral infarction.

Table III shows other diseases in group A possibly predisposing to vascular incidents or disease. It is noted that there was no striking difference between patients with or without vascular events. The

Table II. *Vascular events in patients with brady-tachycardia and in patients with AV block*
Months before or after pacemaker implantation given within parentheses

Type/location of vascular event	No. of pats.	No. of deaths from vascular events	Residual defects: Serious	Residual defects: Slight, none	No. of pats. with vascular events: Before implantation	No. of pats. with vascular events: After implantation
Brady-tachycardia						
Hemiplegia	11	6	3	2	1 (0)	10 (3-2[illegible])
Brain stem	1	–	–	1	1 (0)	–
Transient ischemic attacks	[illegible]	–	–	2	–	2 ([illegible]-4[illegible])
Femoral artery	1		–	1	–	1 ([illegible]4)
Pulmonary artery	3		–	3	2 (48-[illegible]4)	1 (0)
AV block						
Hemiplegia	14	6	4	4	4 (72-1)	12 (1-7[illegible])
Femoral artery	4	–	1	3	1 (170)	3 ([illegible])
Mesenteric	1	1	–	–	–	1 ([illegible])





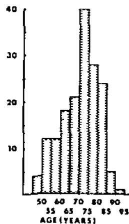


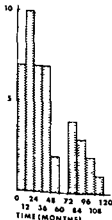
Fig 1 Age distribution *Group A* bradycardia alternating with tachycardia *group B* sinoatrial disease without tachycardia *group C* AV block

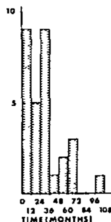
average age of patients who suffered vascular events was somewhat less 66 years than of patients without this complication 72 years

Paroxysmal tachyarrhythmia was unchanged in 4 of the 15 patients with vascular events improved or disappeared in 5 while permanent atrial fibrillation occurred in 6 The corresponding numbers among patients without vascular events were 10 12 and 6 respectively The pacemaker types were fixed rate in 5 QRS triggered in 6 and QRS inhibited in 5 patients at the time of thromboembolism The corresponding numbers in patients without vascular incidents were 10 13 and 5 respectively As regards antiarrhythmic drugs 11 of the 15 patients with vascular events received digitalis and 2 propranolol Of patients without vascular events 19 received digitalis 3 propranolol 1 verapamil and 3 quinidine

In group B 2 of 30 patients aged 72 and 70 years suffered acute cerebrovascular attacks compatible with embolism In one of them the incident occurred 12 months after pacemaker implantation He died suddenly 8 months later The other patient suffered similar attacks 12 and 24 months after pacemaker implantation and died during the last attack One of them had congestive heart failure the other had hypertension and diabetes

The localization and the residual defects of the vascular incidents in group C as well as the time of





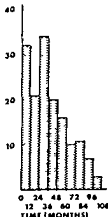


Fig 2 Total duration of pacing Groups as in Fig. 1

Table III *Other diseases in patients with brady tachycardia*

	Pats with vascular events	Pats without vascular events
N	15	27
Age (y)	66 (53–81)	72 (59–86)
Duration of pacing (mo)	39 (3–113)	39 (0–108)
Ischemic heart disease	5	6
Hypertension	2	1
Valvular disease	2	6
Congestive heart failure	3	9
Diabetes mellitus	1	1
Malignant neoplasm	0	1
No other disease	5	15

occurrence in relation to pacemaker implantation are shown in Table II Cerebrovascular incidents occurred in 14 patients 6 of whom died All incidents were compatible with embolism Three of these patients had in addition embolism in a femoral artery One other patient had femoral artery embolism only and another had mesenteric artery occlusion making a total of 16 patients with probable embolic episodes out of 165 Seven patients died and 5 were seriously disabled It is noted that most incidents also in this group took place after pacemaker implantation In 6 of the patients paroxysmal or permanent atrial fibrillation had been recorded previously The total number of patients with supraventricular arrhythmias in group C was 42

Several patients in group C had other diseases which could predispose to vascular disease hypertension valvular disease diabetes mellitus malignancy etc There was however no significant difference in this respect between those who suffered vascular events and those who did not

A comparison between the three groups revealed no significant differences as regards age of patients or the presence of other diseases which might predispose to vascular events Apart from the sex distribution the only striking difference was the high incidence of vascular events compatible with embolism in group A with sinoatrial bradycardia alternating with tachycardia compared with the other groups

COMMENTS

It is difficult to prove that all vascular incidents were due to embolism especially as most events were cerebrovascular The clinical course was compatible with embolism and so were the findings in the two autopsied patients Still as all vascular events were recorded the frequency of embolism could have been overestimated and some patients may have had hemorrhage or thrombosis but it seems unlikely that this can explain the marked differences in the three groups studied The occurrence of alternating atrial tachyarrhythmias and atrial standstill seems to be an important mechanism in thromboembolism as the incidence of embolism in patients with AV block was not influenced by the presence of tachyarrhythmias

Our results support those of Fairfax et al (4) who found evidence of embolism in 16 of 100 patients with chronic sinoatrial disorder All except one of these 16 patients had the brady tachycardia syndrome To our knowledge this is the only other study aimed at exploring the frequency of embolism in various groups of bradycardia Our finding of 35% embolic events in 43 patients with the brady tachycardia syndrome is in accordance with 48% among 21 patients reported by Radford and Julian (7) and 30% among 17 patients reported by Krishnaswami and Geraci (6) On the other hand Eraut and Shaw (3) and Kaul et al (5) did not observe any embolic episodes among 46 and 60 patients respectively with sinoatrial disease The latter number included 38 patients with the brady tachycardia syndrome The patients in these two studies were somewhat younger than ours But it seems unlikely that the difference in age alone could explain the striking difference in occurrence One explanation may be other differences in patient population Sinoatrial disease is a heterogeneous disorder and the criteria for inclusion used by the various authors are not always distinctly given or exactly comparable

It may seem surprising that most vascular events in our study took place after pacemaker implantation In another study (7) most incidents occurred before pacemaker implantation However pacemaker implantation in itself is very rarely associated with embolism either venous or systemic and this discrepancy may be connected with different indications for pacemaker therapy if therapy is early and aggressive embolism may develop after pacemaker implantation if late and conservative it may occur before

All studies so far published have been retrospective and a prospective study is needed with rigid

criteria for inclusion of patients having sinoatrial disease With this reservation it seems reasonable to conclude from our results that anticoagulant therapy should be evaluated in patients with bradycardia alternating with supraventricular tachyarrhythmias

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Electrocardiographic Changes during Total Energy Deprivation (Fasting)

T Theorell J Kjellberg[1] and J Palmblad

From the Department of Medicine Serafimerlasarettet Departments of Medicine IV and VI Sodersjukhuset and the Laboratory for Clinical Stress Research Karolinska Institute Stockholm Sweden

ABSTRACT The effect of 10 days of total energy deprivation (fasting) on ECG reactions was evaluated in 14 healthy normal weight males The heart rate and the ratios between QRS and T wave amplitudes in leads I and II decreased significantly, as did body weight and blood glucose levels The urinary excretion of adrenaline increased It is concluded that other hormonal (e g thyroidal), neural and metabolic mechanisms are of greater significance for the heart than the sympatho-adreno-medullary activity during fasting T wave abnormality without an abnormal Q wave and without other clinical symptoms was noted in one subject on the 8th day of starvation and remained abnormal for more than a year

Previous studies (3, 6 10) have demonstrated that long lasting energy deprivation is associated with ECG changes the most frequent being sinus bradycardia decreased QRS and T wave amplitudes and shifts in QRS and T wave axis The study is intended to throw light on the relations between total energy deprivation (fasting) and various physiologic reactions e g ECG changes

SUBJECTS AND PROCEDURES

The subjects and the experimental procedure have been described in detail elsewhere (7)

Twenty healthy males army officers and soldiers with average weight of 77 2±2 5 kg (mean ± S E M) volunteered (informed consent) to participate in the study After 4 prestarvation days with standardized ordinary food ad libitum 14 subjects were assigned to an experimental (starving) group and 6 to a control group The control group continued on the standard food regimen throughout the study The experimental group was deprived of all food and allowed to drink only non-calorie beverages for the following 11 starvation days In an attempt to avoid dehydration the daily intake of fluid was not allowed to be less than 3 l

Individual serum electrolytes were measured regularly and the fasting subjects were given sodium bicarbonate and potassium chloride accordingly After the 11 days of complete starvation during which the subjects exhibited a mean weight loss of 6 4±0 3 kg (range 8 5–5 4) standard food was gradually re introduced during the last four days (poststarvation period) Throughout the study all subjects took part in routine military training but not in athletics or any other strenuous physical exercises The routines were made equivalent from day to day

ECG recordings were performed in the afternoon on prestarvation day 1 on starvation days 4 8 and 10 and on the second day after discontinuation of starvation A full set of ECG recordings was obtained only from the starving subjects Urine samples for the measurement of catecholamines in this part of the study were obtained from each night s sleeping period Blood samples were obtained for B glucose analyses at 8 00 a m on starvation days 4 7 and 10 All subjects were weighed every morning

METHODS

The *heart rate* was calculated from the ECG recordings Furthermore means were calculated from five consecutive QRS complexes of the ratios between QRS and T wave amplitudes in leads I (inverted T ratio) and II

Catecholamine concentrations in urine Freshly voided urine was acidified to pH 3 and frozen at −20°C until analyzed for catecholamine contents with an Auto-Analyzer fluorescence method (1)

Blood glucose concentrations were measured by a glucose oxidase method (Glox Novum Kabi Stockholm Sweden) and serum electrolytes with a routine method

[1] *Present address* Department of Medicine County Hospital Lidkoping Sweden

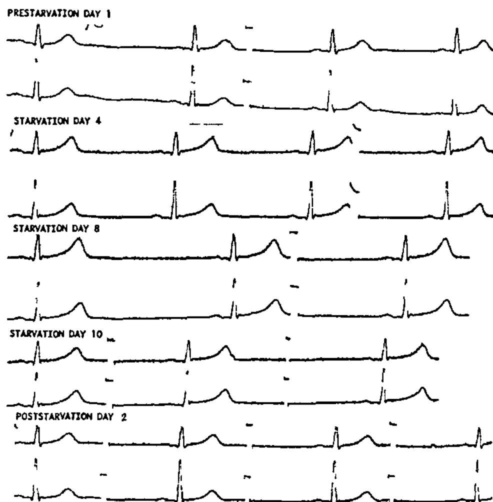


Fig 4 ECG pattern for two of the starving subjects before during and after the starvation period as indicated in the figure

tudes had decreased significantly in leads I and II after 10 days of fasting From the results of previously quoted studies we could assume that absolute T and QRS mean amplitudes both decreased significantly Thus a decrease in the ratio indicates that the T amplitude has decreased significantly *less* than the QRS amplitude Since it was shown that the QRS/T ratio decreased both in lead I an in lead II this shift could not be explained by di: similar rotations of the QRS and T wave axes re

Table I *Significant correlation of changes from the values on day 1 (all correlations were positive)*

	Δ day 8 – day 1	Δ day 10 – day 1
Heart rate	Inverted T ratio in lead I*	
Inverted T ratio in lead I	Heart rate* Inverted T ratio in lead II**	Inverted T ratio in lead II*
Inverted T ratio in lead II	Inverted T ratio in lead I**	Inverted T ratio in lead I*
Blood glucose	Inverted T ratio in lead II*	Inverted T ratio in lead II * Inverted T ratio in lead I*

* = $p<0.05$ ** = $p<0.01$

PRESTARVATION DAY 1

STARVATION DAY 4

STARVATION DAY 8

STARVATION DAY 10

POSTSTARVATION DAY 2

spectively Therefore it could be concluded that a *relative* increase in the repolarization amplitude compared with that of the depolarization has taken place during starvation

An increased excretion of adrenaline in urine was found during the first two thirds of the starvation period The lack of an influence from this enhanced catecholamine excretion which most probably reflects increased blood catecholamine levels on the cardial activities studied here may be explained by a decrease in the sensitivity of the myocardium due to other metabolic and endocrine changes e g prolonged decreased blood glucose and thyroid hormone levels and/or ketosis Some other endocrine changes relevant to this discussion have been reported earlier in an analysis based on the same material (8) For instance a marked fall in serum triiodothyronine levels was observed during the first days of starvation the levels being relatively stable during the last days when they were

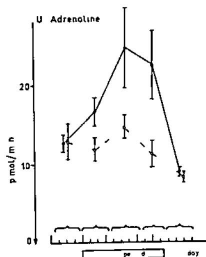


Fig 5 Urinary levels of adrenaline (mean + S E M) ●—● Starving subjects O- O=controls Data are given as the sum of each of the following periods the last three prestarvation nights the first four the four middle and the last three starvation nights and the four poststarvation nights

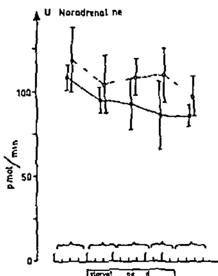


Fig 6 Urinary levels of noradrenaline (mean ± S E M) Data and symbols as in Fig 5

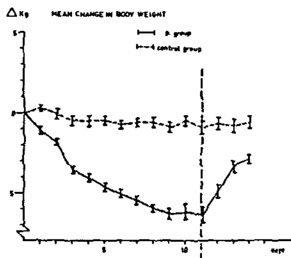


Fig 8 Change in body weight (mean ± S E.M) for the experimental and control groups Shaded area represents the starvation period

close to the hypothyroid range Also serum growth hormone levels increased during the first week returning to normal during the final days of the starvation period Thus the metabolic reactions were different during the last days of starvation as compared with the first week It is of interest to note that changes in ECG dependent variables correlated significantly with changes in blood glucose levels mainly during the last days of starvation

A lowered sensitivity to catecholamines has also been found in hypothyroidism (4 9) and supports

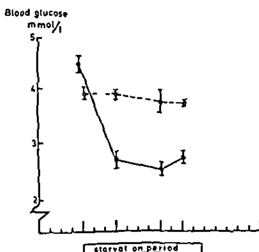


Fig 7 Blood glucose concentrations Symbols as in Figs 5 and 6

the present hypothesis that a decreased sensitivity of the myocardium to the enhanced catecholamine activity may be due to the concomitant hypothyroid state

One interpretation of the observations may be that preserving mechanisms e g an increase in the activity of the parasympathetic nervous system become important particularly after the first week of starvation when catabolism of protein and fat provides the main amount of energy

The influence of changes in calcium and magnesium balance may also be important A negative balance has been reported previously in starvation (2 5) Both magnesium and calcium deficiency in the myocardium may induce ECG changes similar to those observed in the present study

The dramatic T wave changes which were observed in one of the subjects could not be explained clinically

ACKNOWLEDGEMENTS

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The Tromsø Heart Study

Distribution of Serum Cholesterol between High Density and Lower Density Lipoproteins in Subjects of Norse Finnish and Lappish Ethnic Origin

O H Førde D S Thelle N E Miller[1] and O D Mjøs

From the Institutes of Community Medicine Clinical Medicine and Medical Biology University of Tromsø Tromsø Norway

ABSTRACT There are indications that subjects of Lappish ethnic origin have a lower incidence of coronary heart disease than subjects of Norse or Finnish descent, despite their having a similar coronary risk factor status as assessed by serum lipid concentrations, BP and cigarette consumption The possibility that this reflects a more favourable serum lipoprotein pattern in Lapps has been examined in 116 non fasting male subjects of Norse, Finnish and Lappish descent matched for age (mean age 39 years) and level of habitual physical activity No significant differences between the three ethnic groups were observed, however, in either the serum total cholesterol concentration or the distribution of serum cholesterol between the high density (HDL density (*d*) 1 063–1 21 g/ml) and lower density (<1 063 g/ml) lipoprotein classes In view of recent evidence of an association of several coronary risk factors with a low HDL level in other populations the relationship of HDL cholesterol concentration to other variables was explored by stepwise multiple regression analysis As in other communities, HDL cholesterol was inversely related to the *d*<1 063 cholesterol concentration HDL cholesterol showed no significant correlation however, with either relative body weight, cigarette consumption or BP Any relationship of these variables to coronary risk in the Tromsø population must operate, therefore, through mechanisms unrelated to HDL

There is evidence that subjects of Lappish ethnic origin have a lower incidence of clinical coronary heart disease (CHD) than those of the other major ethnic groups of northern Norway the Norsemen and the Finns In an unpublished geographical analysis of mortality among males aged 40–69 years conducted in Finnmark County during 1959–62 the inland areas Kautokeino and Karasjok which are inhabited mainly by Lapps had CHD mortality rates of 4 3 and 3 9 per 1 000 respectively (29) In contrast the coastal areas of Hammerfest and Sør Varanger had mortality rates of 18 8 and 18 0 per 1 000 The reported occurrence of myocardial infarction among the first-degree relatives of subjects who participated in the primary survey of the Tromsø Heart Study (27) showed a corresponding difference between the three ethnic groups only 8% of Lapps reported a family history of MI which could be confirmed whereas the figures for Finns and Norsemen were 16 and 13% respectively

The primary survey of the Tromsø Heart Study showed that a low incidence of CHD among Lapps cannot be explained on the basis of conventional coronary risk factors (27) Thus although Lappish subjects had slightly lower diastolic BPs than Norsemen the converse was true for serum total cholesterol concentration which was on an average 23 mg/dl higher in the Lapps There were no significant differences between the three ethnic groups in serum triglyceride concentration systolic BP or cigarette consumption

There is recent evidence that clinical CHD is more strongly related to the distribution of serum cholesterol among the different lipoproteins than to the serum total cholesterol concentration CHD prevalence increasing with increasing low density lipoprotein (LDL density (*d*) 1 006–1 063 g/ml)

Address for reprints O H Førde Institute of Community Medicine University of Tromsø N 9000 Tromsø Norway

[1] *Present address* Baker Medical Research Institute Melbourne Victoria 3181 Australia

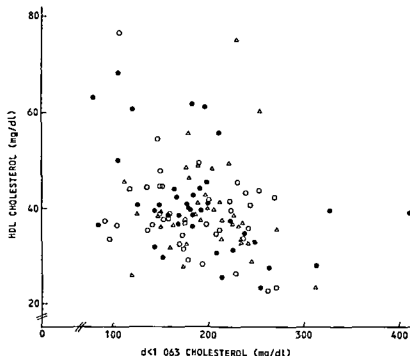


Fig 1 Relationship between the concentrations of cholesterol within serum lipoproteins of densities <1 063 and 1 063–1 21 g/ml (HDL) in adult males of Norse (O) Finnish (Δ) and Lappish (●) ethnic origin

DISCUSSION

These data indicate that there are no major differences in the distribution of serum cholesterol be een the high density and lower density lipoproclasses in adult males of Norse Lappish and nish ethnic origin living in the Tromsø area Any ower incidence of clinical CHD among Lappish subjects would not therefore result from a more favourable serum lipoprotein pattern during adult life These observations do not exclude the possibility however that ethnic differences exist in lipoprotein pattern during childhood and adolescence which influence the development of CHD in later life Consideration must also be given to the fact that the currently available statistics on CHD in the three ethnic groups were provided by the 1959–62 mortality in Finnmark (29) and by the family histories obtained during the primary survey of the Tromsø Heart Study (11) As the two ethnic minorities of the Tromsø region the Lapps and the Finns are now well integrated with the Norse majority both culturally and socioeconomically it is possible that recent changes in living habits may have altered their serum lipoprotein patterns and incidence rates These questions may be answered by the repeat survey in Finnmark due to start in 1977 in which also HDL cholesterol will be determined in addition to several other coronary risk factors

In view of the close integration of the three ethnic groups in the Tromsø area the present study would be expected to detect only genetically as opposed to environmentally determined differences in lipoprotein pattern There are few known examples of racial differences in serum lipoproteins which cannot be explained on the basis of environmental factors Although several ethnic groups with a low incidence of CHD (relative to western societies) have been shown to have a low concentration of lower density and/or a high concentration of high density lipoproteins this has usually been attributed to living habits (1 4 16) A notable example are the Eskimos of rural Greenland in whom low levels of pre β- and β lipoproteins and high levels of α lipoproteins (corresponding respectively to VLDL LDL and HDL) appear to result from a diet low in saturated and rich in unsaturated fatty acids and in males also from a high level of habitual physical activity (1) An exception however may be provided by Black Americans whose serum lipoprotein pattern differs from that of White Americans partly it seems for genetic reasons (28) Within racial groups genetic factors are known to influence serum lipoprotein levels (14)

The inverse relationship of HDL cholesterol con

centration to the $d<1\ 063$ cholesterol and serum triglyceride concentrations accords with similar observations in other communities (7 8 9 10 15 16 18 20 22 25) This has reflected predominantly *a negative correlation between the concentrations* of HDL and the triglyceride rich VLDL although in some populations HDL cholesterol concentration is also negatively correlated with LDL cholesterol (16 20 25) Even in communities in which LDL concentration shows no significant correlation with the total HDL concentration a negative correlation has nevertheless been demonstrated with the HDL subfraction HDL_2 (d 1 063–1 125 g/ml) (13 18) This is of interest in view of other evidence that it is *mainly* the HDL_2 concentration which shows a sex difference and which is reduced in hypertriglycerid aemia obesity and other conditions associated with increased CHD risk (2 13 18)

The absence of any significant relationship in the present data between HDL cholesterol concentra tion and either relative body weight or cigarette consumption contrasts with the results of studies in other communities in which HDL concentration has been reduced in obesity (8 10 13 22) and in cigarette smoking (21) The presence of a low HDL level in these situations has been postulated as providing one mechanism for the increased coronary risk with which they are associated (15 17) Any relationship of CHD to obesity or cigarette smoking in the Tromsø population however must operate largely through other mechanisms

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The Effect of Smoking on Selected Coronary Heart Disease Risk Factors in Middle-Aged Men

Jan Eriksen and Sven Chr Enger

From Medical Department B Rikshospitalet Oslo and the Department of Clinical Chemistry Drammen Hospital Drammen Norway

ABSTRACT Associations between smoking and selected factors supposed to promote coronary heart disease (CHD) were studied in 1832 healthy men, 809 of whom were smokers Triglycerides were 7% higher in smokers the heaviest smokers had the highest levels Unfavourable changes were not seen in the other parameters in smokers There was no trend indicating an influence of smoking on total and HDL cholesterol in the *individuals who had abstained* from smoking for a few hours just before the examination Body mass index and weight blood pressure and resting heart rate were lower in smokers whereas heart volume, glucose tolerance, thyroid function and ECG findings did not differ significantly in smokers and non smokers The present study indicates that smoking promotes the development of CHD independently of the commonly accepted CHD factors

Of the commonly accepted risk factors for coronary heart disease (CHD) smoking is claimed to be one of the most important in promoting premature CHD morbidity and mortality (17) We have previously shown that smoking was strongly associated with impaired lung function and poor physical performance and possibly also with increased coronary atheromatosis as judged from coronary angiographic findings in presumably healthy middle aged men (8)

The present study aims at assessing possible associations between smoking and a selected number of commonly accepted CHD risk factors by examining the CHD-free men in the same study population

STUDY POPULATION AND METHODS

A total of 1832 men without any symptoms or signs of CHD were studied They participated in a cardiovascular survey at Medical Department B Rikshospitalet To obtain a presumably healthy population individuals were only accepted if the following diseases and disorders were not recorded by the factory health officer or disclosed on arrival for the survery Known CHD other known heart disease hypertension under treatment with drugs diabetes mellitus malignancy disorders of the locomotor system preventing a bicycle exercise test and miscellaneous diseases (advanced lung disease renal disease liver disease etc)

The survey covered 2014 men aged 40–59 years from five major companies and governmental institutions i e 86% of the eligible men The detailed modes of selection are presented elsewhere (6 9 10) Altogether 182 men were excluded from the present study because of latent CHD or chronic chest pain disclosed through the examination program Although not a random sample our population is a fairly representative group of healthy middle aged men

The individuals met at 7 30 a m after at least 12 hours abstinence from smoking and fasting for an examination program (6) which in all individuals included case history physical examination X ray of heart and lungs phonocardiography resting ECG and a near maximal bicycle exercise test with in- and postexercise ECG recordings serum cholesterol triglycerides and protein bound iodine I v glucose tolerance test was performed in all but 42 (7) and HDL cholesterol was assessed in a random subsample of 247 of the 1832 men (5)

The individuals were divided into current non smokers and current smokers and subdivided as never smokers previous smokers and individuals smoking 1–9 10–19 and ≥20 cigarettes daily regardless of inhalation habits and years of smoking Sixty two pipe or cigar smokers were included as cigarette smokers (1 g tobacco = 1 cigarette)

Resting ECG was described paying particular attention

Table I *CHD risk factors in relation to smoking habits among 1832 presumably healthy middle aged men*
x=mean value S E =standard error of x

Parameter	Current non smokers (n=1 023) x	S E	Current smokers (n=809) x	S E	Level of significance
Body weight (kg)	77 4	0 31	75 7	0 34	***
Body mass index*	1 006	0 0035	0 989	0 004	***
Systolic BP (mmHg)	130 5	0 54	128 6	0 60	*
Diastolic BP (mmHg)	87 8	0 32	86 0	0 36	***
Resting heart rate (beats/min)	62 1	0 30	60 4	0 34	***
Absolute heart volume (ml)	774	4 5	772	5 0	NS
Relative heart volume (ml/m² BSA)	400	2 0	402	2 2	NS
Total cholesterol (mmol/l)	6 56	0 037	6 67	0 041	NS
Triglycerides (mmol/l)	1 40	0 024	1 50	0 027	**
HDL cholesterol (mmol/l) (n=247)	1 40	0 030	1 37	0 032	NS

NS=not significant (p>0 05) *p<0 05 **p<0 01 ***p<0 001
* Body weight (kg)/(Height (cm)−100)

to the Minnesota Code (11) aberrations 1 2 and 1 3 (Q wave) 5 2 5 3 and 5 4 (T wave) 2 1 (left axis deviation) and 3 1 (left ventricular hypertrophy)

The relations between smoking habits and the following parameters were assessed Body weight body mass index total cholesterol HDL cholesterol triglycerides systolic and diastolic BP resting heart rate absolute and relative heart volume glucose tolerance (k value) thyroid function (PBI) and the ECG codes mentioned above

The statistical analyses were made by means of one way analysis of variance multiple t tests and χ^2 dis bution using conventional levels for statistical sig icance (10)

RESULTS

Table I shows values for various physiological and laboratory parameters in current non smokers and current smokers Current smokers have significantly lower body weight body mass index BP and resting heart rate and higher triglyceride level Heart volume total cholesterol and HDL cholesterol were not significantly different Smoking did not influence the glucose tolerance and the thyroid function (results not shown)

Table II *Height/weight indices and lipid levels in relation to smoking habits in 1832 healthy middle aged men*

All parameters corrected for differences in age (age correction factors obtained from regression analysis of the various parameters with regard to age changes) x and S E as in Table I LS 1=Level of significance for differences in mean values between never smokers and the various subgroups of smokers LS 2=LS 1 between previous smokers and the various subgroups of smokers

Parameter			Never smokers (n=453)	Previous smokers (n=570)	Smokers 1–9 cig /d (n=309)	10–19 cig /d (n=399)	≥20 cig /d (n=101)
Body weight (kg)	x	(LS 1)	76 7	77 9	74 6 **	76 5	75 5
	S E	(LS 2)	0 46	0 41	0 56 ***	0 49	0 98 *
Body mass index	x	(LS 1)	1 004	1 008	0 983 **	0 998	0 974 *
	S E	(LS 2)	0 0053	0 0048	0 0064 ***	0 0057	0 0113
Total cholesterol (mmol/l)	x	(LS 1)	6 49	6 63	6 61	6 73 **	6 59
	S E	(LS 2)	0 055	0 049	0 067	0 059	0 117
Triglycerides (mmol/l)	x	(LS 1)	1 38	1 40	1 44	1 52 **	1 59 *
	S E	(LS 2)	0 036	0 033	0 044	0 039	0 077
HDL cholesterol* (mmol/l)	x	(LS 1)	1 41	1 40	1 39	1 35	1 42
	S E	(LS 2)	0 043	0 044	0 048	0 047	0 100

* Determined in 247 (65 63 52 55 and 12) individuals in the 5 subgroups

Table III *Smoking habits in relation to minor Q wave aberrations in resting ECG among 1 832 presumably healthy middle aged men*

Smoking habit	Normal resting ECG (Minnesota Code 1 0)	Probable myocardial infarction (Minnesota Code 1 2–)[a]	Possible myocardial infarction (Minnesota Code 1 3–)[a]	Total
Never smokers	439	1	13	453
Previous smokers	557	3	10	570
Smoking 1–9 cig /d	300	1	8	309
Smoking 10–19 cig /d	390	2	7	399
Smoking ≥20 cig /d	100	0	1	101
Current non smokers	996 (97 4%)	4 (0 4%)	23 (2 2%)	1 023
Current smokers	790 (97 6%)	3 (0 4%)	16 (2 0%)	809
Total	1 786	7	39	1 832

Ref no 14

In Table II comparisons of the lipid parameters body weight and body mass index are presented after further subdivisions of the material according to smoking habits Previous smokers have the highest body weight and body mass index whereas the heaviest smokers have the highest triglycerides There is no trend showing an influence of smoking on cholesterol although those smoking 10–19 cigarettes daily have marginally higher serum cholesterol than never smokers HDL cholesterol did not differ significantly between groups Resting heart rate systolic and diastolic BP were significantly higher in previous smokers whereas the heaviest smokers had the lowest values (results not shown)

Table II shows that the prevalence of Q wave aberrations was almost identical in current non smokers and smokers and the different smoking habit subgroups have essentially the same proportion of individuals with normal ECGs or Q wave changes Neither did the distribution differ significantly between current non smokers and smokers or smoking habit subgroups for T wave changes left axis deviation or ventricular hypertrophy (χ^2 test $p>0$ 10 for all distributions—results not shown)

DISCUSSION

CHD morbidity and mortality are increased in smokers (17) Smoking may be an independent CHD risk factor or may influence the development of CHD through interference with other risk factors

Of the parameters examined in the present study definitely unfavourable changes in smokers were only observed for serum triglycerides Triglycerides showed a consistent gradient within the smoking habit subgroups with a 15% higher level in the heaviest smokers than in never smokers However differences of this size hardly explain the CHD-promoting effect of smoking (3)

Although cholesterol was slightly higher in smokers than in non smokers and significantly higher in those smoking 10–19 cigarettes daily there was no consistent pattern of cholesterol changes with smoking habits Elevated cholesterol in smokers has been reported from the same geographic area previously (11 13)

Possibly the present modes of selection ruled out individuals with high cholesterol and heavy smoking because of a CHD-promoting effect of this combination (8) *Acute* increase in serum cholesterol immediately after smoking has been reported (4) Thus the possible chronic cholesterol increasing effect of smoking may only be assessed in individuals who have not smoked in the few hours just before the blood samples were taken In our study almost all smokers had abstained from smoking for more than 12 hours and all for more than 3 hours

The other changes observed in the present subjects should rather diminish than increase the risk of developing CHD in smokers The study confirms that smokers are leaner whereas previous smokers gain weight after stopping smoking (16) Smokers also had a lower resting heart rate which if any thing ought to diminish the risk of CHD (2) Non smokers had higher BP—in conformity with other studies in which the individuals had not smoked prior to the examination (12 16 17)

Neither glucose tolerance thyroid function nor

Table I *CHD risk factors in relation to smoking habits among 1832 presumably healthy middle aged men*
x=mean value S E =standard error of x

Parameter	Current non smokers (n=1 023) x	S E	Current smokers (n=809) x	S E	Level of significance
Body weight (kg)	77 4	0 31	75 7	0 34	***
Body mass index[a]	1 006	0 0035	0 989	0 004	***
Systolic BP (mmHg)	130 5	0 54	128 6	0 60	*
Diastolic BP (mmHg)	87 8	0 32	86 0	0 36	***
Resting heart rate (beats/min)	62 1	0 30	60 4	0 34	***
Absolute heart volume (ml)	774	4 5	772	5 0	NS
Relative heart volume (ml/m² BSA)	400	2 0	402	2 2	NS
Total cholesterol (mmol/l)	6 56	0 037	6 67	0 041	NS
Triglycerides (mmol/l)	1 40	0 024	1 50	0 027	**
HDL cholesterol (mmol/l) (n=247)	1 40	0 030	1 37	0 032	NS

NS=not significant (p>0 05) *p<0 05 **p<0 01 ***p<0 001
[a] Body weight (kg)/(Height (cm) − 100)

to the Minnesota Code (11) aberrations 1 2 and 1 3 (Q wave) 5 2 5 3 and 5 4 (T wave) 2 1 (left axis deviation) and 3 1 (left ventricular hypertrophy)

The relations between smoking habits and the following parameters were assessed Body weight body mass index total cholesterol HDL cholesterol triglycerides systolic and diastolic BP resting heart rate absolute and relative heart volume glucose tolerance (k value) thyroid function (PBI) and the ECG codes mentioned above

The statistical analyses were made by means of one way analysis of variance multiple t tests and χ^2 distribution using conventional levels for statistical significance (10)

RESULTS

Table I shows values for various physiological and laboratory parameters in current non smokers and current smokers Current smokers have significantly lower body weight, body mass index BP and resting heart rate and higher triglyceride level Heart volume total cholesterol and HDL cholesterol were not significantly different Smoking did not influence the glucose tolerance and the thyroid function (results not shown)

Table II *Height/weight indices and lipid levels in relation to smoking habits in 1832 healthy middle aged men*

All parameters corrected for differences in age (age correction factors obtained from regression analysis of the various parameters with regard to age changes) x and S E as in Table I LS 1=Level of significance for differences in mean values between never smokers and the various subgroups of smokers LS 2=LS 1 between previous smokers and the various subgroups of smokers

Parameter			Never smokers (n=453)	Previous smokers (n=570)	Smokers 1–9 cig /d (n=309)	Smokers 10–19 cig /d (n=399)	Smokers ≥20 cig./d. (n=101)
Body weight (kg)	x	(LS 1)	76 7	77 9	74 6 **	76 5	75 5
	S E	(LS 2)	0 46	0 41	0 56 ***	0 49	0 98
Body mass index	x	(LS 1)	1 004	1 008	0 983 *	0 998	0 974
	S E	(LS 2)	0 0053	0 0048	0 0064 * *	0 0057	0 0113 *
Total cholesterol (mmol/l)	x	(LS 1)	6 49	6 63	6 61	6 73 *	6 59
	S E	(LS 2)	0 055	0 049	0 067	0 059	0 117
Triglycerides (mmol/l)	x	(LS 1)	1 38	1 40	1 44	1.52 **	1 59
	S E	(LS 2)	0 036	0 033	0 044	0 039	0 077
HDL cholesterol[a] (mmol/l)	x	(LS 1)	1 41	1 40	1 39	1 35	1 42
	S E	(LS 2)	0 043	0 044	0 048	0 047	0 100

[a] Determined in 247 (65 63 52 55 and 12) individuals in the 5 subgroups

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Electroencephalographic Prediction of Anoxic Brain Damage after Resuscitation from Cardiac Arrest in Patients with Acute Myocardial Infarction

M Møller B Holm E Sindrup and B I yager Nielsen

From the Departments of Medicine B and Clinical Neurophysiology Odense University Hospital Odense Denmark

The short term prognostic value of electroencephalography (EEG), carried out n the days after cardiac arrest, was evaluated in a study of 185 patients with acute myocar infarction together with an episode of clinical arrest The individual EEGs were classified n a 5 grade scale Of the 89 patients who survived, had signs of anoxic brain damage 96 patients ied, 76 as a result of cerebral anoxia Only 2 patients out of the total of 72 for whom the first EEG classified as grades III-V The EEGs of both patients were recorded within a few hours after cardiac arrest None of the patients with an EEG f grade I died of cerebral anoxia while all degrees of rain damage were otherwise observed in connection EEGs of both grades I and II It is concluded at an EEG of grades III-V indicates a fatal out provided it has been recorded more than 24 ours after the cardiac arrest A grade III-V EEG is recorded within 24 hours after a cardiac arrest uld be repeated some days later It is not possible the basis of a single EEG, to predict the extent of brain damage

- use of external heart massage and DC defibril in patients with cardiac arrest has considera y increased the possibility of successful resuscita ion

It is of considerable clinical interest to find thods for evaluating the patient s chances of urviving the acute phase and also of predicting the of any anoxic brain damage It is known from ier electroencephalographic studies that a re uction in consciousness produces changes in the ectrobiological activity of the cerebrum also that the character and duration of these changes in the electroencephalogram (EEG) are related to the cause of the reduction in consciousness In 1962 Pampiglione (5) used the EEG changes to determine the prognosis with regard to occurrence of brain damage in a group of young children resuscitated following cardiac arrest of varying genesis Different forms of EEG grading have been employed (2 3 6 7) in order to predict a fatal course in patients with cerebral anoxia of varying causes In these studies the EEGs have in the main been recorded within the first 24 hours after an anoxic episode which was most frequently the result of clinical cardiac arrest

In the present investigation of 185 patients with acute myocardial infarction (AMI) and an episode of clinical cardiac arrest the value of EEG recorded on the days after the cardiac arrest has been studied as a predictor of the short term prognosis We assume that the EEG changes first become representative with regard to the short term prognosis at this period

PATIENTS AND METHODS

The present investigation concerns a consecutive series of 187 patients admitted to the Coronary Care Unit (CCU) Odense University Hospital over a period of five years The patients fulfilled the following three criteria 1) AMI according to the WHO criteria (12) 2) an episode of clinical cardiac arrest in connection with or during hospitalization in the CCU and 3) the recording of at least one completed EEG

The series initially consisted of 149 men and 38 women the sex ratio being 4 1 and the average age 63 years The corresponding figures for all the patients with AMI admit-

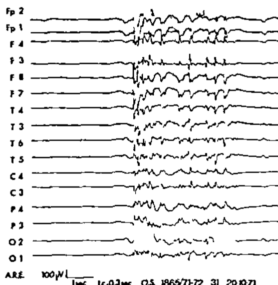


Fig 5 Case 31 A 71 year old man with cardiac arrest outside hospital Period of anoxia more than 5 min EEG less than 24 hours later showed universal paroxysmal bursts of irregular low frequency activity of 1–3 cps mixed with spikes followed by isoelectric periods with severe cortical suppression The paroxysmal bursts were accompanied by universal seizures The patient died 10 days after the cardiac arrest of sequelae following cerebral anoxia without regaining consciousness EEG grade IV

of memory at the time of discharge possibly ned with a few neurological sequelae These pa were normally unable to resume their work but able look after themselves *Major sequelae* Considerable intellectual impairment Severe neurological sequelae The patients were unable to manage even the most simple work and required considerable nursing *Death as a result of sequelae following cerebral anoxia* All of these patients died either without recovering consciousness or in a few cases after they had recovered consciousness but in whom cerebral anoxia must be considered the main cause of death *Death as a result of cardiac arrest* Patients who having recovered consciousness died of a new attack of cardiac arrest and who had no severe neurological/intellectual sequelae at the time of death *Death from other causes* Death from for example pneumonia without severe neurological/intellectual sequelae at the time of death *Death from uncertain causes* The main cause of death was not ascertained

RESULTS

The percentage of patients with ECGs of grades I–II decreases with increasing age 12 (67%) of the patients between 40 and 49 years of age against 26 (58%) of the 70–79-year-old patients This difference is not statistically significant ($p>0.5$ χ^2 test with Yates correction)

About half of all cases of cardiac arrest occurred outside the hospital the great majority of the other half in the emergency room or the CCU i e in departments fully equipped and ready to take immediate care of such patients Table II shows that in 100 patients with a period of anoxia of 5 min or less the EEGs are classified as grades I–II while this applies to only 5 patients with a period of anoxia of more than 5 min The difference is statistically significant ($p<0.001$ χ^2 test)

The primary EEG diagnosis was ventricular fibrillation in 161 patients asystolia in 17 while 7 patients had regained spontaneous heart action before an EEG was recorded

Table III shows the time that elapsed between cardiac arrest and the EEG recording in relation to the EEG grading An EEG was recorded within the first 24 hours in 19% of the patients The patients who regained consciousness shortly after the attack of cardiac arrest often had the EEG recorded several days later

In order to exclude a number of factors influencing either the EEG classification or the neurological/intellectual condition at the time of discharge the patients were divided into two main groups A) 138 patients without any other known cerebral affection than that caused by the actual anoxia and without any new episode of cardiac arrest followed by successful resuscitation during the admission B)

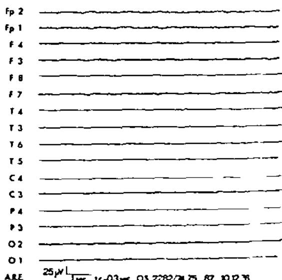


Fig 6 Case 87 A 41 year-old man with cardiac arrest in the emergency room Period of anoxia a few seconds EEG 3 days later showed no cortical activity The patient died of sequelae after cerebral anoxia EEG grade V

Table IV *Outcome in the patients of groups A and B*
c a = Cardiac arrest o c = other causes c u = cause unknown

EEG grade	Sequelae				Dead			
	No	Minor	Major	Coma	Anoxia	c a	o c	c u
Group A (n=138)								
I	34	2	3			3	1	
II	19	3	4		2	4	4	
III					11			
IV					15			
IV*					29			
V					4			
Total	53	5	7	0	61	7	5	0
%	38	4	5	0	44	5	4	0
Group B (n=47)								
I	11	1				2	1	
II	7	1	1	1	5	1	1	2
III			1					
IV					3			1
IV*			1		5			
V					2			
Total	18	2	3	1	15	3	2	3
%	38	4	7	2	32	7	4	7

47 patients with previous cerebral disease and/or a new episode of cardiac arrest with resuscitation during the admission

Table IV shows the outcome in the two groups Out of the 185 patients 89 survived and 96 died 76 of them from anoxic brain damage A total of 71 patients were discharged without neurological/intellectual sequelae seven with minor ten with major sequelae while one patient was comatose

Of the patients in each group 38% were discharged without sequelae while the total percentages of survivors were 47% and 51% in groups A and B, respectively In group A 44% died of anoxic brain damage against 32% in group B The difference is not statistically significant ($p<0.3$ χ^2 test)

In group A 79 (57%) of the patients had an EEG of grades I–II against 34 (72%) in group B The difference is not statistically significant ($p<0.1$ χ^2 test) There is a very pronounced prognostic demarcation between patients with an EEG of grades I–II and III–V only two with the latter grades survived and both of them belonged to group B The remaining patients with an EEG classified as grades III–V died of anoxic brain damage except one patient with uncertain cause of death

All grades of anoxic brain damage were seen in grades I–II though no patient with grade I EEG died of such damage

The two survivors with EEG gradings III–V are briefly described below

Patient 28 a 39-year-old man who had previously been healthy He suffered cardiac arrest outside hospital external heart massage and mouth to-mouth ventilation being commenced immediately by a layman On arrival at the emergency room a few minutes later the ECG showed ventricular fibrillation and resuscitation was continued according to the general principles of the department The circulation was re-established after approximately 30 min He remained in a coma for three days and then gradually recovered consciousness An episode of ventricular fibrillation occurred 15 days after admission and was converted to sinus rhythm by DC defibrillation The patient was transferred to a psychiatric hospital with major sequelae

An EEG recording less than 18 hours after the cardiac arrest was graded as IV* while a new EEG four days later was graded as I

Patient 65 a 54-year-old man who had had cerebral apoplexy with right sided hemiparesis some 10 years prior to the cardiac arrest He was still spastic in the extremities of the right side The patient developed cardiac arrest while at home The ambulance attendants commenced resuscitation approximately 6 min later The ECG showed asystolia on arrival at the emergency room After 20 min the circulation could be re-established and the patient was

Table V Outcome in relation to the time at which consciousness was regained and the EEG grading
o c = Other causes including cardiac arrest c u = cause unknown

EEG grade	Conscious on the first day						Conscious on the following days						Unconscious			
	Sequelae			Dead			Sequelae			Dead			Dead			
	No	Minor	Major	Anoxia	o c	c u	No	Minor	Major	Anoxia	o c	c u	Coma	Anoxia	o c	c u
I–II	65	7	2		14	1	6		6	1	3		1	6		1
III–V									2	1				68		1

comatose for approximately 24 hours whereafter he gradually regained consciousness He was severely impaired intellectually and required considerable nursing at discharge one month later

The EEG taken on the day of admission was classified as grade III while a new EEG two weeks later was graded as II

Table V shows the 185 patients classified in relation to the time at which they eventually recovered consciousness It can be seen that continued coma for more than 24 hours after the cardiac arrest is not necessarily an indication for a fatal outcome A total of 19 patients recovered consciousness more than 24 hours after the cardiac arrest and six of them survived without sequelae eight with major [illegible]lae while five died

In 42 cases the first EEG was supplemented by a [illegible] EEG on the days that followed This was *particularly the case in patients with signs of anoxic* brain damage Only two patients (nos 28 and 65) shifted from grades III–V to grades I–II one patient from grades I–II to grades III–V this patient died of cerebral anoxia

DISCUSSION

A reduction in the degree of consciousness results in changes in the electrobiological activity of the cerebrum and the character of the EEG changes and their duration depend on the cause of the reduction

Hockaday et al (3) applied a five-degree rating scale to 39 patients with cardiac arrest or apnea Of *the 25 with an EEG of grades IV–V only one sur*vived The EEG of this patient was recorded during the first 24 hours following the cardiac arrest and a new EEG taken three days later was classified as grade II

In an investigation of 120 children under 10 years of age who had been resuscitated after cardiac arrest from varying causes Pampiglione and Harden (6) found that a four grade assessment of *the EEG was useful for early evaluation of brain* damage The optimal time at which to record the EEG is stated to be 2–12 hours after the cardiac arrest when serial recordings are used

In our investigation there is a pronounced prognostic difference between patients with an EEG of grades I–II and III–V The length of the anoxic period appears to be the decisive factor The two survivors with EEGs of grades III–V belonged to group B Another characteristic of both these patients was that the EEG had been recorded within the first 24 hours of the cardiac arrest and that a new EEG some days later was classified as grades I–II These findings suggest that the prognostic value of an EEG belonging to grades III–V is best *when recorded at the earliest on the second day after the cardiac arrest*

Our study demonstrates that a single EEG recording is not a sufficient basis for predicting the extent of any anoxic brain damage in the surviving patients Visual grading of the recorded EEG was *employed in the present investigation The 5 grade* scale is a modification of The London Hospital Rating System (7 8)

EEG patterns with the characteristics of alpha rhythm in comatose patients are found in our study Such patterns have been described with brain stem strokes and with hypoxic encephalopathy after cardiopulmonary arrest (4 10) When evaluating the EEG we therefore took the *patient s state of consciousness into consideration* In this way EEGs with alpha rhythm in comatose patients could be classified as grade IV*

Among the patients of Prior (7) 66 are comparable with our group A and 24 with our group B *while the other patients did not suffer from cardiac*

arrest Both in that work and in a study of the same group of patients by Binnie et al (2) it is suggested that the individual EEG should be evaluated discriminately upon the basis of 46 characteristics in order to avoid difficulties in classification and to obtain greater precision with regard to the prognosis In the investigation of Binnie et al (2) the precision with regard to death/survival in 41 patients without previous cerebral disease or other conditions that could be considered as having an influence on the EEG is stated to be 99% The group only includes patients who either died of histologically verified anoxic brain damage or who survived without intellectual or neurological defects In a group of 33 patients—corresponding more or less to our group B—the prognostic precision was considerably lower

A simple EEG recording technique has been employed in the present investigation together with simple grading in order to study the value of a procedure that can be employed in any cardiological department which can obtain the services of a specialist in clinical neurophysiology

Willoughby and Leach (11) have used the neurological condition one hour after the cardiac arrest as the prognostic parameter in a series of 48 patients The patients who at the time of the recordings showed no reaction or who only reacted to painful stimuli in a reflex fashion either died or survived with intellectual impairment Patients without these neurological findings one hour after cardiac arrest survived without sequelae

Our study does not completely support these findings inasmuch as six of the 96 patients who were comatose after the first 24 hours regained consciousness and survived without sequelae Similarly Bell and Hodgson (1) in a study of 133 patients in a coma after cardiac arrest found that 18 patients who were comatose after 24 hours recovered consciousness and could be discharged Six of these patients had their full intellectual faculties The study did not include EEG recordings

On the basis of the present investigation we conclude that in patients resuscitated after clinical cardiac arrest caused by AMI a visual evaluation of a single EEG recorded at the earliest 24 hours after the cardiac arrest is of considerable prognostic value If the EEG belongs to grades III–V the patient will not survive Should the EEG fall into grades I–II the prognostic value is less but it must be emphasized that in this group we have seen patients regain consciousness after the first 24 hours and survive without sequelae An EEG recorded within the first 24 hours after the cardiac arrest and falling into grades III–V should be repeated one or two days later

ACKNOWLEDGEMENT

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Third Degree Atrioventricular Block, Chronic Progressive External Ophthalmoplegia and Pigmentary Degeneration of Retina

Case Report and Survey of the Literature

Erik la Cour Petersen

From the Department of Cardiology University Hospital Århus Denmark

ABSTRACT A woman was noted to have chronic progressive external ophthalmoplegia, pigmentary degeneration of retina and third degree AV block She was admitted to hospital because of syncopes and was successfully treated with a permanent pacemaker Since 1958, 12 similar cases have been noted Three of 7 patients without pacemaker treatment died, and 5 were successfully treated with pacemaker The disturbances in AV conduction are not thought to be a mere coincidence to the ocular disorder Cardiomyopathy has been suggested

It is well known that cardiac involvement is an often recognized feature of different kinds of neuromuscular disorders

Since 1958 AV conduction disturbances have been *described in* patients with cronic progressive external ophthalmoplegia (CPEO) and pigmentary degeneration of retina AV block is a life threatening complication to a harmless disorder of the ocular muscles

CASE REPORT

A 24-year-old female sewer had for 12 years noticed a gradually developing ptosis on both eyes She and her family never thought it abnormal or unusual and never went to see a doctor

There was a family history of hemicrania and from earliest childhood the patient had had attacks of headache accompanied by vomiting and photophobia

The patient had for 6 months suffered from spells of unconsciousness when she was admitted to a local hospital in March 1974 after such an attack She had third degree AV block with a ventricular rate of 20/min and was immediately transferred to our Department of Cardiology

Examination at the time of admission showed a woman of small stature exhausted and apathetic ECG revealed third degree AV block with a ventricular rate of 24/min (Fig 1)

A transvenous right ventricular pacing catheter type Elema 588 was inserted and connected to an external pacemaker A few days later an Elema 154 demand pacemaker was inserted subcutaneously on the left side of thorax

At the primary examination a grade 2 soft systolic murmur was heard at the left sternal border The murmur disappeared during artificial pacing Chest X ray on admission showed increased heart size (13/22 cm) One week later heart size was normal (10 5/22 5 cm)

Neurological and ophthalmological examinations showed bilateral ptosis (Fig 2) and severely restricted movements of the eyes both in vertical and horizontal plane and in convergence Ophthalmoscopy showed a thin atrophic retina with scattered spots of pigment The visual field was normal with preserved central vision contrary to what is typically found in the classic retinitis pigmentosa The skeletal muscles were somewhat poorly developed but muscle strength and reflexes were within normal limits During the pacemaker operation a sample of skin and muscle was removed for examination Histological examination showed no signs of neuromuscular disorder No psychological test was carried out but the IQ was estimated to be at the lower normal limit The patient was shy and timid and due to this ocular muscle biopsy and spinal fluid examination were not carried out

Laboratory data including complete RBC investigation electrolytes in plasma renal function S-GOT LDH antistreptolysin titer antistreptococcal hyaluronidase reaction LE cell preparation antinuclear factor and thyroid function were all within normal limits

The patient has been followed in our Out Patient Clinic for 30 months There have been no spells of fainting or dizziness The AV block has been permanent and the

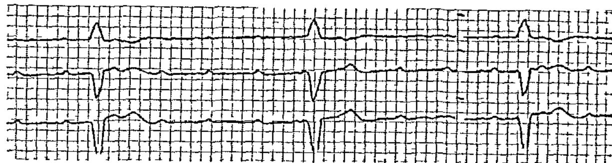


Fig 1 ECG showing third degree AV block with a ventricular rate of 24/min

pacemaker is functioning There have been no cardiopulmonary symptoms and no other signs of cardiac involvement

COMMENTS

Heart disease is common in primary myopathies (9 12 15) Post mortem examinations have shown myocardial changes similar to those found in skeletal muscles James (4) also found degenerative changes in the vessels supplying the sinus node and the AV node

CPEO starts with ptosis in the first or second decade of life followed by increasing immobility of extraocular muscles The pupil is spared Ex for weakness of the facial muscles in some involvement of skeletal muscles cannot be CPEO is often associated with a peculiar pigmentation of retina different from retinitis pigmentosa (5)

Von Graefe was the first to describe the disorder in 1868 (2) and since then a number of similar cases have been published At first it was considered a disorder of the nervous system but in 1946 Sandifer (13) demonstrated with a rectus externus biopsy that it seemed to be a myopathy rather than a disorder of the nervous system His patient had bradycardia and bundle branch block and so he was the first to suggest a possible heart involvement in CPEO In 1958 Kearns and Sayre (6) published two cases of CPEO and pigmentary degeneration of retina complicated with third degree AV block Since then a few similar cases have been published Including the present case the total number is 13

Table I shows relevant clinical data from all the cases published with CPEO pigmentary degeneration of retina and third degree AV block the latter verified at least once Cases with only lesser disturbances of conduction (5) are omitted Females and males are equally represented Ocular symptoms in most cases preceded cardiac symptoms by several years but the latter were the most common reason for admission to hospital

Table II shows histopathological findings in some of the cases Skeletal muscle biopsy or necropsy disclosed abnormalities in two cases of 6 examined Ocular muscle biopsy or necropsy showed changes similar to those found in primary myopathy in all 4 cases examined In all 3 fatal cases post mortem examination of the heart was performed In two of them (2 6) no abnormalities were disclosed but in the third case (5) examination showed enlarged hyperchromatic muscle nucleus focal subendothe



Fig 2 The patient with apparent bilateral ptosis and restricted movements of the eyes

Table I *Clinical data on 13 patients with chronic progressive external ophthalmoplegia pigmentary degeneration of retina and third degree AV block*

Case no author year ref no	Age (y)	Sex	Duration of eye symptoms (y)	Duration of heart symptoms	Cause of admission	Treatment of AV block	Death	Follow up
1 Kearns & Sayre 1958 (6)	34	♂	14	8 years	Syncope	None	−	None
2 Kearns & Sayre 1958 (6)	17	♂	14	2 months	Syncope	Epinephrine	+	None
3 Jager et al 1960 (3)	13	♂	3	None	Ocular disease	Ephedrine isoproterenol	+	None
4 Kearns 1965 (5)	35	♀	19	2 weeks	Ptosis syncope	None	−	None
5 Kearns 1965 (5)	34	♂	17	17 years	Cardiac incompensation	None	−	None
6 Kearns 1965 (5)	17	♂	14	2 months	Syncope	None	+	None
7 Drachman 1968 (1)	16	♀	8	1 year	Ocular disease syncope	Isoproterenol	−	None
8 Drachman 1968 (1)	27	♂	14	4 months	Syncope	Pacemaker	−	None
9 Ross et al 1969 (11)	23	♂	10	6 months	Syncope	Pacemaker	−	None
10 Shastri et al 1971 (14)	17	♀	11	2 days	Syncope	Pacemaker	−	20 months
11 Morriss et al 1972 (8)	16	♀	5	None	Ocular disease	Pacemaker	−	None
12 Pilling & Nanton 1974 (10)	23	♀	3	Few *hours*	Syncope	Pacemaker	−	20 months
13 Present author 1977	24	♀	12	6 months	Syncope	Pacemaker	−	30 months

lial fibrosis and a slightly thickened endocardium The changes were believed to be responsible for the conduction disturbances Six cases all diagnosed after 1965 received pacemaker treatment Follow up data are available on 3 patients The pacemaker treatment had eliminated the tendency to syncope Without pacemaker treatment the prognosis seems to be serious Before the pacemaker era 3 patients out of 7 died due to syncope Treatment with pacemaker seems to improve the prognosis though follow up information is available on half of the cases only

It may be questioned whether the cases published until now (Table I) represent a clinical entity However other features than AV block and ocular involvement were present in all cases The patients were small and slightly built with poorly developed skeletal muscles IQ was estimated to be at the lower end of the normal range Increased protein in the spinal fluid was found in all patients who had this examination Chromosome analysis turned out normal No familiar predisposition has been demonstrated

The ocular disease without cardiac involvement seems to be rather infrequent Kiloh and Nevin (7) found reports of 99 cases It is generally regarded as an inconvenient but harmless disorder but when complicated with third degree AV block the condition becomes dangerous It demands prompt pacemaker therapy In patients with ptosis or other signs of ocular myopathy ECG must be recommended in order to disclose AV conduction distur

Table II *Histopathological findings in patients with chronic progressive external ophthalmoplegia pigmentary degeneration of retina and third degree AV block*

Case no	Skeletal muscle examination	Ocular muscle examination	Histological examination of the heart
1	None	None	None
2	Necropsy normal	Necropsy myopathy	Necropsy normal
3	Necropsy normal	Necropsy myopathy	Necropsy normal
4	Biopsy myopathy	None	None
5	None	None	None
6	None	Necropsy myopathy	Necropsy*
7	None	None	None
8	Biopsy normal	Biopsy myopathy	None
9	Biopsy nearly normal	None	None
10	None	None	None
11	None	None	None
12	None	None	None
13	Biopsy normal	None	None

* Enlarged hyperchromatic muscle nucleus focal subendothelial fibrosis and a slightly thickened endocardium

, before progression to third degree block
... et al (8) showed that His bundle recording can be of some aid in doubtful cases

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Mınoxıdıl ın Severe Hypertensıon

Marttı Oka and Mattı Makela

From the Department of Internal Medıcıne Central Hospıtal Jyvaskyla Fınland

ABSTRACT Mınoxıdıl has been admınıstered to 16 patıents with severe hypertensıon and renal failure In every patıent the ındıcatıon for mınoxıdıl treatment was resıstance to conventıonal drugs The final dose of mınoxıdıl was 2 5–30 mg (average 20) and ıt was combıned with a β blockıng agent and a dıuretıc (or dıalysıs) The therapy was gıven for 1–27 months (average 12) The average supıne BP fell from 200/130 to 164/96 mmHg and the uprıght BP from 200/120 to 152/90 mmHg No hypotensıve reactıons occurred In most patıents the progressıon of hypertensıve organ changes was arrested No major vascular complıcatıons have occurred durıng the 16 years of treatment Prıcklıng of the skın and hırsutısm were common sıde effects The other sıde-effects observed were oedema ın five patıents and development of latent dıabetes ın three In four patıents mınoxıdıl treatment was dıscontınued for followıng reasons successful reconstructıon of the renal artery after stenosıs, renal transplantatıon, severe oedema and hırsutısm The rısk of hırsutısm ıs a contraındıcatıon to prolonged mınoxıdıl admınıstratıon ın most female patıents Mınoxıdıl ıs especıally ındıcated ın uncontrolled renal hypertensıon

Mınoxıdıl (2 6-dıamıno-4 pıperdınopyrımıdıne 1 oxıde) ıs a new vasodılator wıth antıhypertensıve propertıes It has been used ın severe hypertensıon when conventıonal drugs have faıled (1 4 6 7 8 9 10) But besıdes reducıng hypertensıon mınoxıdıl causes salt retentıon and cardıac stımulatıon (2) Thus ıt ıs almost always essentıal to supplement therapy wıth a dıuretıc and a β blockıng agent (3)

In thıs paper we descrıbe our experıences ın usıng mınoxıdıl wıth success to control BP ın 16 patıents who were refractory to the maxımally tolerated doses of conventıonal antıhypertensıve agents

Requests for reprınts to Dr M Oka Central Hospıtal SF-40620 Jyvaskyla 62 Fınland

PATIENTS AND METHODS

Mınoxıdıl was admınıstered to 16 patıents (11 men and 5 women) wıth severe hypertensıon and renal faılure (Table I) Theır ages ranged from 17 to 63 years (average 45) and the duratıon of hypertensıon from a half to 20 years (average 4 7) Fıve patıents were undergoıng chronıc haemodıalysıs for 2–12 months (average 7) and all were candıdates for kıdney transplantatıon

Inıtıally the dosage was adjusted ın hospıtal The patıents were then seen weekly untıl the BP had fallen to a stable level Thereafter check ups were made monthly In every patıent the ındıcatıon for mınoxıdıl therapy was resıstance to conventıonal drugs Varıous combınatıons of antıhypertensıves (ıncludıng hydralazıne but not prazosıne) ın the maxımal tolerated doses had been ın adequate ın controllıng hypertensıon The number of antıhypertensıve drugs at the start of mınoxıdıl therapy was 2–6 (average 3 5)

The hypertensıon was severe as ındıcated by a sustaıned supıne BP of 195–260/110–160 mmHg despıte treatment Of the 16 patıents 15 belonged to the WHO class III of hypertensıon ECG revealed left ventrıcular hypertrophy (LVH) ın 14 and X ray an enlarged heart ın 11 Fourteen patıents had congestıve heart faılure and were receıvıng dıgıtalıs and two had angına The funduscopıc findıng was of grade II–IV ın nıne (Keıth Wagener–Barker classıficatıon) and renal faılure (elevated s creatınıne level) was present ın 9 Three patıents had prevıous hıstorıes of hypertensıve crısıs or cerebral vascular accıdent

In seven patıents the hypertensıon was of the essentıal type ın eıght renal and ın one renovascular The dısease process leadıng to renal faılure was chronıc glomerulonephrıtıs ın five and pyelonephrıtıs prımary amyloıdosıs and polycystıc degeneratıon ın one patıent each One patıent had stenosıs of the renal artery and was gıven mınoxıdıl as preoperatıve treatment

Pretherapeutıc and follow up studıes ıncluded a blood pıcture lıver enzymes s Na s K s-creatınıne s-creat

Table I *Historical characteristics of the patient population*
LVH=left ventricular hypertrophy CHF=congestive heart failure CVA=cerebrovascular accident

Pat no	Age (y)	Sex	Etiology*	Duration of hypertension (y)	WHO grade of hypertension	Dialysis/nephrectomy	No of antihypertensive drugs at onset	Indication for minoxidil therapy[b]	Eye grounds (grade)
1	40	♂	E	1	III	−/−	6	C	III
2	53	♂	F	13	III	−/−	3	C	II
3	58	♂	F	3	III	−/−	3	C	I
4	47	♀	R	4	III	−/−	2	C S	I
5	54	♀	RV	2	I	−/−	5	C	II
6	59	♂	E	10	III	−/−	3	C S	I
7	53	♀	R	5	III	−/−	4	C	II
8	26	♂	R	1	III	+/+	3	C	III
9	50	♂	E	2	III	−/−	3	C	II
10	63	♂	R	20	III	−/−	3	C S	IV
11	35	♂	R	0 5	III	+/−	4	C	III
12	48	♀	E	1	III	−/−	5	C S	I
13	17	♂	R	0 5	III	+/−	2	C	I
14	21	♂	R	0 5	III	+/−	3	C	I
15	40	♂	E	3	III	−/−	4	C S	I
16	48	♀	R	0 5	III	+/−	3	C	III

* E=essential R=renal RV=renovascular [b] C=control failure S=side effects of other drugs

inine clearance fasting blood glucose urinary sedi ment ECG chest X ray and an ophthalmoscopic exami nation When latent diabetes was diagnosed in patient 1 glucose tolerance test was added to the test battery

Supine and prone BPs heart rate and weight were re at every check up Measurements of blood volume and cardiac output were performed in a few cases using a radionuclide method (5)

The regime is shown in Table II Our goal was to gradu ally reduce and ultimately discontinue the previous drugs and to institute the triple drug medication minoxi dil+a diuretic+a β blocking agent This was achieved in

Table II *Minoxidil and concomitant antihypertensive drug therapy*

Pat no	Duration (mo)	Dose (mg/day) Minoxidil	Propranolol	Timolol	Furosemide	Hydrochlorothiazide + amiloride (50 mg+5 mg) tab	Spironolactone
1	27	30	240			1	
2	21	20	160			1	
3	20	25	480			1	
4	20	2 5	60		80		
5	1	25	480				100
6	19	25		45		1	
7	2*	30	120		2 500		
8	1*	25	120				
9	18	10	60				100
10	17	30	120		360		
11	12	15	480		120		
12	11	30	480		375		75
13	9	7 5	120		1 500		
14	7	15	120		500		
15	5	25	360			1	
16	3*	5	240				
Mean	12	20	245				

* Therapy discontinued

VH/ HF	Renal failure	Attacks of hypertensive crisis	CVA
+/+	–	+	–
/+	–	–	–
/+	–	–	–
/–	+	–	–
/	–	+	–
/+	–	–	–
/+	+	–	–
/+	+	–	–
/+	–	–	–
/+	+	–	–
/+	+	–	–
/+	+	–	+
/+	+	–	–
/+	+	–	
/–	–	–	–
/+	+	–	–

every case Minoxidil was initiated in a dose of 2 5 mg If the BP response was not adequate the dose was increased after three days to 2 5 mg twice daily The dose of minoxidil was increased by 2 5 mg at intervals of a few days according to the response until the BP reached a stable lower level The drug was given in equally divided doses twice daily (11) The doses of the diuretic and the β blocking agent were adjusted individually to control fluid retention and reflex tachycardia

RESULTS

Minoxidil and concomitant antihypertensive drug therapy
(Table II)

The duration of treatment varied from one to 27 months (average 12) The final daily dose of minoxidil was 2 5–30 mg (average 20) and of propranolol 80–480 mg In addition diuretics were administered to 14 patients in a widely varying dosage

Blood pressure response to minoxidil therapy
(Table III)

Minoxidil depressed the BP level successfully The average supine BP fell from 200/130 to 164/96 mmHg The corresponding change in the upright BP was from 200/120 to 152/90 mmHg There were no orthostatic hypotensive reactions In two patients (nos 2 and 3) the upright systolic BP was clearly lower than the supine BP In every patient the tachycardia induced by minoxidil was controlled by β blocking drugs

Effect of minoxidil on LVH heart volume and cardiac output
(Table IV)

ECG signs of LVH increased in five and decreased in two patients (ST segment and T wave changes) Heart size increased in 8 patients but it should be remembered that the series included several pa

Table III *Blood pressure (mmHg) and pulse response (beats/min) to minoxidil therapy*

Pat no	Before minoxidil			After minoxidil		
	BP supine	BP upright	Pulse supine	BP supine	BP upright	Pulse supine
1	200/130	190/140	70	150/105	140/100	72
2	225/130	165/120	54	120/ 80	115/ 75	60
3	230/130	210/120	60	155/100	115/ 95	70
4	230/130	–	80	120/ 90	115/ 75	80
5	210/160	260/140	74	155/ 85	1[illegible]0/ 75	65
6	200/130	200/130	70	160/ 90	165/ 95	60
7	260/130	230/125	62	[illegible]00/100	–	60
8	220/140	190/130	124	190/110	170/ 95	90
9	210/125	180/115	68	150/100	150/ 95	68
10	210/120	–	80	180/100	175/ 85	64
11	225/130	–	80	150/ 90	–	70
12	235/130	–	76	190/110	–	76
13	195/120	–	78	140/ 80	–	80
14	225/125	–	60	190/ 90	–	75
15	200/130	180/130	64	130/ 95	125/ 90	72
16	260/110	–	100	190/100	170/ 80	80
Mean	220/130	200/120	75	164/ 96	152/ 90	71

Table IV *Effect of minoxidil treatment on LVH heart volume and serum creatinine level*

	Decreased	Unchanged	Increased	Average change
LVH	2	9	5	
Heart size (±15%)	3	5	8	+42 cm^3/m^2
Serum creatinine (±10%)*	1	6	4	+16 μmol/l

* Patients on dialysis are not included

tients with advanced renal failure A sudden transient increase in cardiac output was measured in our first patient in whom the start of β blocking therapy was delayed In the other patients cardiac output did not change significantly during the trial

Serum creatinine level and fundi
(Tables IV and V)

An increase in the serum creatinine level was observed in four patients and a decrease in one (the patients on dialysis excluded) The eyeground status improved in three patients and was unchanged in the others

Side effects of minoxidil and reasons for discontinuing therapy

Thirteen patients complained of slight prickling of skin which was followed by hypertrichosis growth of hair was noticeable (13 patients) especially on the arms face and eyebrows In one female patient (no 16) hirsutism necessitated discontinuation of therapy Only two women are still receiving minoxidil patient 2 is on a small dose (2 5 mg) there has been no other alternative to control the BP of patient 12 and she has accepted the cosmetic annoyance Oedema appeared in five patients and was the reason for discontinuing the therapy in one patient (no 7) The other reasons for discontinuation of minoxidil administration have been successful renovascular surgery and successful renal transplantation (patients 5 and 8)

We have not found any previous reports on derangement of glucose metabolism during minoxidil treatment In three of our patients (nos 1 3 and 12) the fasting blood glucose level was elevated 10 20 and 27 months after onset respectively and the glucose tolerance test gave abnormal results The test had given normal results in patients 1 and 3 before the trial It had not been performed in patient 12 before the trial The pretreatment fasting blood glucose level was normal in all

DISCUSSION

The main effects of minoxidil are dilatation of peripheral arteries and reduction of peripheral vascular resistance The haemodynamic consequences are tachycardia increased cardiac contractility stroke volume and cardiac output The metabolic consequences are increased secretion of renin and catecholamine secondary hyperaldosteronism and retention of sodium and water

The reflex increase in pulse rate was easily controlled by concomitant administration of propranolol or timolol in all our patients and the water retention by diuretics in all but one

The BP response to minoxidil was favourable All the patients who are still on therapy are normotensive or nearly normotensive although before taking minoxidil their BP was uncontrolled in spite of a multidrug regimen

Two patients were successfully pretreated with minoxidil before renovascular surgery or renal transplantation The treatment was especially valuable in refractory renal hypertension Five of the patients were undergoing long term haemodialysis

No major complications of hypertension occurred during the 16 years of treatment ECG changes in ST segment and T wave increased in a few cases and so did roentgenological heart size Cardiac output was unaffected by minoxidil In a few patients a gradual deterioration of renal function could not be arrested Favourable changes in the fundi were observed in a few cases and deterioration in none

Table V *Fundi before (B) and after (A) minoxidil treatment*

Grade	B	A
I	7	10
II	4	5
III	4	1
IV	1	0

In two cases treatment was discontinued because of side effects the reasons were severe oedema and hirsutism The hirsutism which developed in 13 out of 16 patients did not annoy the men (they were proud of it) but was really troublesome in the women The face was covered with downy hair In our opinion, prolonged administration of minoxidil to women should be restricted to the few patients for whom no alternative therapy can be found Minoxidil did not have virilizing effects Prickling of the skin and hypertrichosis may be due to increased skin circulation Derangement of glucose metabolism was observed in three cases The fasting blood glucose level increased and the glucose tolerance test revealed latent diabetes This complication of minoxidil has not been reported previously The therapy has been continued in these cases

Our overall opinion of minoxidil therapy is definitely favourable We have been able to control severe hypertension in many patients in whom conventional therapy had failed We feel that minoxidil has been life saving in some cases as no major vascular complications occurred during the 16 years of therapy In refractory renal hypertension minoxidil has been especially valuable It is an alternative to nephrectomy and can be used preoperatively and to treat graft rejection

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Increased Serum Levels of Immunoglobulins in Untreated and Treated Essential Hypertension

I Relation to Blood Pressure

Bent Østergaard Kristensen

From Medical Department P Randers City Hospital University Hospital of Århus Randers Denmark

ABSTRACT In 164 consecutive patients with essential hypertension, serum immunoglobulin (Ig) levels of IgA, IgG and IgM classes were determined, using single radial diffusion techniques, and compared with 80 healthy normotensive subjects without any family history of hypertension Of 80 untreated and 84 treated patients, IgA and/or IgG were significantly increased in 40 % and 37 %, respectively IgG correlated positively to BP in untreated patients ($p<0\ 0008$), as well as in insufficienctly treated males ($p<0\ 004$) No correlations were found between Ig and duration of hypertension The increase in Ig was not associated with any particular drugs A family history of hypertension was found in 19 6 % of the patients with elevated Ig and in 9 7 % of those with normal Ig ($p<0\ 10$) The study provides further evidence for involvement of immune mechanisms in essential hypertension, and suggests a possible genetic predisposition

Serum levels of IgG have been reported to be elevated in patients with a mean arterial blood pressure (MAP) above 130 mmHg irrespective of the etiology of the hypertension (1) In a mixed group of untreated and treated patients with essential hypertension serum levels of IgG and/or IgA have been reported to be increased in about 30 % of the cases but without any correlations to BP (11) The findings were interpreted as being secondary to vascular damage On the other hand an immune pathogenetic mechanism in the development of hypertension has also been proposed (8) The natural cell turnover or cell death was in some cases thought to release antigens and it was suggested that autoantibodies by causing vascular damage induce an increase in peripheral resistance and raise BP

However irrespective of inducing mechanisms an association of serum levels of the Ig with BP might be expected in untreated hypertension as reduction of BP with drugs does not necessarily imply a restoration of any possible morphological vascular changes In the present study determinations of serum levels of Ig have been performed in 1) a consecutive series of untreated patients with essential hypertension 2) treated patients from the Out Patient Clinic and 3) a group of healthy normotensive individuals with the same composition by age and sex as the groups of hypertensive patients and without any family history of hypertension

STUDY POPULATION

Untreated patients

A request was sent to the general practitioners in the region emphazising the interest in untreated patients with hypertension Within nine months 89 patients suspected of having hypertension were referred to the Out Patient Clinic and included to the study according to the following criteria 1) The hypertension should be classified as essential (see Methods) 2) Absence of diseases which could influence the Ig level in the patient and his family (allergic autoimmune liver and infectious diseases within the previous three months) these diseases were excluded according to standard medical examinations 3) No use of contraceptive pills Of the 89 patients referred 14 were excluded Nine women used contraceptive pills two patients had bronchial asthma one had estival fever one glomerulonephritis and one renovascular hypertension Five patients three males and two females were already attending the Out Patient Clinic The final group consisted

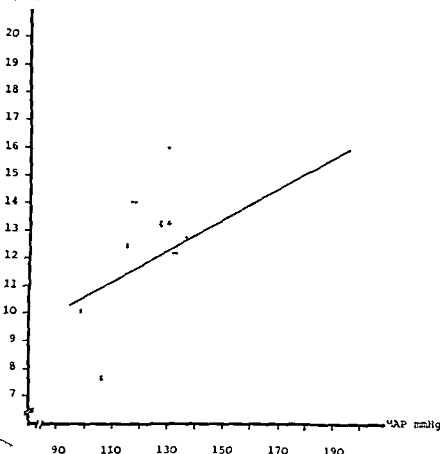


Fig 1 Correlation of IgG with mean arterial BP (*MAP*) in 60 untreated patients with essential hypertension (45 ♂ 35 ♀) $Y = 0.54x + 5.2$ $r = 0.37$ $p < 0.0008$

er groups of treated patients no correlations were found between IgG and MAP No correlations to IgA or IgM were encountered in any of the hypertensive patients There was no correlation between any Ig group and MAP in the controls

Albumin concentration in the untreated males was significantly higher than in the controls (Table II) This was not the case in any other groups neither did the HMT differ in any groups although there was a tendency towards higher values in the hypertensive patients To further evaluate a possible influence of haemoconcentration in the patients the Ig/HMT Ig/albumin and albumin/HMT ratios in the hypertensive patients and controls were compared The Ig (A and G)/HMT and the Ig (A and G)/albumin ratios were found to be 10–40% higher than in the controls with exactly the same pattern as for the mean values (not shown) For IgM and albumin/HMT the average ratios were one in all groups

The upper normal limit for each Ig class was calculated as the mean +2 S D in the controls so that 2 5% of the normal individuals will show levels above these limits Table III shows the number of hypertensive patients with Ig above these limits The number of patients with IgM levels above the limit is very close to the expectation of 2 5% In contrast the number of untreated patients with IgA and IgG levels above these limits varied from 22 2 to 28 6% and in the treated from 15 2 to 33 3% Of the 60 untreated patients 32 (40%) had an elevation of either IgA or IgG (not shown) six males and three females had both classes elevated Of the 84 treated patients 31 (37%) had these elevations four males and three females had both Ig classes elevated (neither shown)

Table IV presents the results from a comparison of untreated patients with and without elevated Ig MAP was higher albeit insignificantly (p 0 07) in the patients with elevated Ig It should be noted that mean age did not differ between the two groups Albumin concentrations and HMT values were also quite similar as was the duration of the hypertension About 73% had a known duration of less than

Table III *Hypertensive patients with Ig levels above mean + 2 S D in 80 normotensive controls*

	IgA (g/l)		IgG (g/l)		IgM (g/l)	
	♀	♂	♀	♂	♀	♂
Mean + 2 S D in 80 controls (38 ♀ 42 ♂)	3 0	3 0	14 0	13 5	3 4	2 9
Untreated pats (35 ♀ 45 ♂)						
n	9	10	10	12	1	1
%	25 7	22 2	28 6	26 6	2 9	2 2
Treated pats (33 ♀ 51 ♂)						
n	11	13	5	9	2	1
%	33 3	25 5	15 2	17 6	6 1	2 0

24 months In the treated patients (not shown) MAP was lower in the group with elevated Ig but still not significant ($p>0$ 10) Neither in these patients did the clinical data or protein concentrations differ between the two groups The duration of the hypertension was less than 24 months in 50% of these patients In none of the hypertensive groups was a correlation found between Ig and duration As the drugs used in treatment might have caused orthostatic hypotension the statistical analyses were performed using supine MAP In the treated males supine MAP was significantly higher than erect but not in any other groups and the principal findings were not altered No association of Ig with any particular drugs was found

A family history of hypertension was found in 19 6% of the hypertensive patients with increased Ig against 9 7% of those with normal Ig ($p<0$ 10) Information about the family could not be obtained in seven untreated and eight treated patients

DISCUSSION

The present study demonstrates an increase in IgA and/or IgG in about 40% of untreated as well as of treated patients with essential hypertension the duration of which was less than 24 months in 73% and 50% respectively IgG correlated positively to BP in untreated patients and in insufficiently treated males A tendency towards a genetic predisposition was encountered

The serum levels of Ig in the present control group agree well with those of others using the single radial diffusion technique (2 3) The frequency of elevations in hypertensive patients also agrees with previous reports (1 11) but in addition the present study demonstrated a similar increase in untreated as well as in treated patients A relation of IgG to severe hypertension (MAP ≥130 mmHg) has been demonstrated previously and found in treated patients with hypertension of various etiologies (1) In the present study mean IgG in the treated males with MAP ≥130 mmHg did not differ from that of the controls whereas their IgA was significantly higher In all other groups with MAP ≥130 mmHg both IgA and IgG were significantly higher than in the controls our results thus seem to confirm the findings of Ebringer and Doyle (1) In a study of 84 patients with essential hypertension Olsen et al (11) also demonstrated a pattern of increased IgA and IgG but without any correlation to BP In that study untreated and treated patients were not evaluated separately and the present finding that the mean values of Ig were highest in the treated patients with MAP ≤115 mmHg may explain this lack of a correlation especially as a highly significant correlation was found between IgG and BP in the treated males with MAP ≥130 mmHg and in the untreated patients The correlation coefficients (r) were however not very high but this may be due to the wide individual variations of these proteins also found in normal subjects

The cause of the demonstrated raised Ig levels in essential hypertension is not known As patients with diseases known to influence the Ig were excluded from this study and similar frequencies of elevations were found in untreated and treated patients neither these disorders nor the drugs used in treatment of high BP could be the cause The possi

Table IV *Age known duration of hypertension mean arterial BP (MAP) and serum levels of Ig in untreated hypertensive patients with and without elevated IgA and/or IgG (mean±S E M)*
HMT=haematocrit

	Elevated Ig	Normal Ig
No of pats	32	48
Mean age (y)	41	38
Months of known duration	18	15
Range	1–92	1–120
MAP (mmHg)	129±3	122±2
IgA (g/l)	3 1±0 2	1 9±0 1
IgG (g/l)	14 1±0 4	10 7±0 3
Albumin (g/l)	44 1±0 7	44 6±0 6
HMT	44 ±0 7	45 ±0 5

• $p<0$ 07

bility of a haemoconcentration known to occur in hypertension seems to be eliminated by the findings of increased Ig/HMT and Ig/albumin ratios in the hypertensive patients compared with normotensive controls This finding can be explained either by increased production of Ig or by decreased degradation though the latter seems a more remote possibility (3)

Several groups have reported on involvement of immunological factors in hypertension (5 10 12 13) and the demonstration of mononuclear cells in the hypertensive damaged vessel walls in patients with essential hypertension seems to fulfill the requirements for a production of antibodies against the arterial walls However the antigens have not yet been identified but both the organ specific and the non-organ specific immunity have an elevation of Ig in common

At this stage of the study it is not possible to elucidate whether the increased levels of Ig are secondary to vascular damage or whether an immune pathogenetic mechanism is involved in the development of hypertension as in both cases one would expect an association between the Ig and the severity of the disease It is noteworthy however that untreated and treated patients showed a similar increase in IgA and IgG and that the duration of the pertension was shorter in patients with elevated Nikbin et al (9) have reported on an association serum levels of IgA and IgG but not of IgM with the histocompatibility antigen HLA B27 In this study a higher frequency of a family history of hypertension was observed among the patients with raised Ig which may suggest a genetic ability to react against vascular injuries with increased formations of Ig, and a study of the HLA antigen frequency in the present patients and controls is in progress

In the prospective part of this study we may be able to further evaluate the clinical implications of the present findings However it can be concluded that immune mechanisms are involved at an early stage of essential hypertension

ACKNOWLEDGEMENTS

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Autoantibodies in Untreated and Treated Essential Hypertension I

B Østergaard Kristensen and P L Andersen

From Medical Department P Randers City Hospital University Hospital of Århus Randers and the Institute of Medical Microbiology University of Århus Århus Denmark

ABSTRACT Using the indirect immunofluorescence method, autoantibodies (AB) of IgG and IgM classes were investigated in sera from 161 consecutive patients with essential hypertension and compared with those from 78 healthy normotensive subjects of the same composition by age and sex, and without any family history of hypertension The frequency of one or more AB was as high in 78 untreated (15 3%) as in 83 treated patients (13 0%) and was in the controls 9 0% (n s) In the treated patients the AB were associated with heart involvement ($p<0$ 02) In the untreated patients, antinuclear antibodies of IgG class were associated with BP ($p<0$ 01) and fundus grading ($p<0$ 005) A family history of hypertension was found in 23 5% of the hypertensive males with AB and in 9 0% of the normotensive males ($p<0$ 10) These results are discussed in relation to reports of an association of AB with cardiovascular diseases It is concluded that the presence of AB in essential hypertension is not necessarily due to drug induction

In population studies autoantibodies (AB) have been found to be associated with cardiovascular morbidity and mortality and suggested as being markers of vascular lesions (14) Whether the occurrence of AB is secondary to vascular damage or whether immune mechanisms are involved in the development of these diseases is still being discussed (15) As hypertension is one of the main risk factors in the development of cardiovascular diseases AB might be expected to be more frequent in hypertensive patients than in normotensive subjects and possibly associated with target organ involvement irrespective of inducing mechanisms

Several groups have demonstrated an increased incidence of AB in relation to drugs used in treatment of high BP (3–6 9 18) The present study therefore comprised an investigation of AB in the sera from a consecutive series of both untreated and treated patients with essential hypertension and from a group of healthy normotensive individuals

STUDY POPULATION

Altogether 239 males and females aged 16–68 years were investigated and consisted of the following three groups

Group 1 Patients with untreated essential hypertension including borderline hypertension This group comprised 45 males aged 19–65 years (mean 40) and 33 females aged 16–61 years (mean 39)

Group 2 Patients with established and treated essential hypertension This group comprised 50 males aged 35–65 years (mean 54) and 33 females aged 26–68 years (mean 50) Methyldopa was given to 82% in dosages of 500–2 700 mg daily mean 1 600 mg for an average of 10 months Hydralazine was given to 37% in dosages of 40–300 mg daily mean 96 mg (15 months) Eight patients received propranolol up to 800 mg daily but none were treated with practolol and none had any clinical signs of skin rashes ocular lesions or liver damage

Group 3 Healthy normotensive subjects This group comprised 41 males aged 18–59 years (mean 39) and 37 females aged 18–59 years (mean 39) Originally 164 patients and 80 controls were included in the study but serum samples were used for other immunological assays in two untreated (one of each sex) and one treated male and in one male and one female control

METHODS

Hypertension was defined according to age (13) the lower limit being a systolic BP of 150 mmHg or a diastolic of 90 mmHg on three different occasions Only patients with essential hypertension based on physical examination urine sediment serum electrolytes and i v urography were included Information on the duration of hypertension was obtained either from the patients doctors or previous hospital admissions Evidence of a family history

Table I *Titers and incidences of tissue antibodies in patients with essential hypertension and normotensive controls with same sex and age ranges*

ANA = antinuclear antibodies SMA = smooth muscle antibodies PA = gastric parietal cell antibodies GA = rat glomerular antibodies N = no of total groups *n* = no of subgroups

	Untreated pats (N=78)	Treated pats (N=83)	Controls (N=78)
ANA	5 (6 4 %)	7 (8 4 %)	4 (5 1 %)
Titer range	20–160	20–640	20–40
SMA	5 (6 4 %)	3 (3 6 %)	1 (1 3 %)
Titer range	20–40	20–160	20
PA	2 (2 6 %)	2 (2 4 %)	1 (1 3 %)
Titer range	40–640	20–60	20
GA	0	0	1 (1 3 %)
Titer range			20
Incidence of at least one autoantibody (%)			
Males + females	15 4	13 3	9 0
Females	12 2 (n=33)	6 1 (n=33)	8 1 (n=37)
Males	17 7 (n=45)	18 0 (n=50)	9 8 (n=41)

of hypertension among first degree relatives was gained from a questionnaire The healthy individuals had normal BP ECG urine sediment and no signs of atherosclerotic diseases and no family history of hypertension None of the participants had signs of autoimmune liver or infectious diseases according to standard medical procedures None of the women used contraceptive pills The details concerning selection procedures and exclusion criteria are published elsewhere (11)

BP was measured after 20 min at rest using a sphygmomanometer Diastolic BP was recorded at the disappearance of sound (Korotkoff phase V) BP on the day of admission was used even if higher or lower values had been recorded earlier (10) The same specially trained nurse performed all the recordings ECG was recorded with nine leads Ischaemia was defined as T wave null or negative in I/II or V_6 left ventricular hypertrophy as the sum of S in V_1 and R in $V_{5/6}$ ≥ 35 mm (WHO) Using the method of Musshoff et al (16) heart volume was determined in 141 of the patients within three months of admission All patients had their ocular fundi examined

A serum sample was obtained and stored at −18°C for a period of up to 12 months as the investigation of the sera from both hypertensive patients and controls was carried out simultaneously The AB were demonstrated by the indirect immunofluorescence method using 4 μm thick unfixed cryostat sections of rat stomach and kidneys as antigens (2) The following antibodies were investigated Antinuclear antibodies (ANA) reacting with nuclei of renal tubular cells Smooth muscle antibodies (SMA) reacting with gastric smooth muscles Glomerular antibodies reacting with rat glomeruli Parietal cell antibodies reacting with gastric parietal cells Mitochondrial antibodies reacting with the cytoplasm of both gastric parietal cells and renal tubular cells The sera were investigated for antibodies of the IgG and IgM classes by means of monospecific fluorescein isothiocyanate conjugated anti-human immunoglobulins (Wellcome England) The molar fluorescein protein ratios of the conjugates were between 2 and 4 The conjugates were used in a dilution corresponding to an antibody content of between 1/4 and 1/8 U/ml The sera were tested in doubling dilutions starting with 1 20 and the slides were read blindly

Statistics Student s *t* test for unpaired observations and the χ^2 test for heterogeneity Two-sided tests were performed throughout

RESULTS

The titres and the frequencies of the AB in the three groups studied are given in Table I In both untreated and treated patients the AB titres were higher than in the controls In both groups the AB occurred more often than in controls (not significant) MTA were not demonstrated in any groups

From Table II it can be seen that the treated patients were about a decade older than the untreated but in neither group did mean age differ between patients with and without AB The duration of hypertension was shorter in the patients with AB than in those without

The occurrence of one or more AB in relation to some clinical parameters is indicated in Table III The untreated patients with mean arterial BP (MAP) ≥130 mmHg and with fundus grade II or more had AB more often than those with MAP <130 mmHg and fundus grade 0–I (n s) None had more than grade II changes The occurrence of AB was independent of ECG changes in this group In treated patients the frequency of AB was similar in

Table II *Age and duration of hypertension in hypertensive patients with and without one or more autoantibodies (AB) (mean and range)*

	Untreated pats		Treated pats	
	+AB	−AB	+AB	−AB
No of pats	12	66	11	[illegible]
Age (y)	41 (19–56)	44 (20–61)	56 (35–61)	52 (24–65)
Duration (mo)	5 (1–24)	18 (1–192)	28 (2–60)	42 (1–192)

Table III *Occurrence of one or more autoantibodies in relation to clinical data in 78 untreated and 83 treated patients with essential hypertension*

MAP=mean arterial BP (diastolic BP + 1/3 of pulse pressure) I=ischaemia LVH=left ventricular hypertrophy Fundus grade after Keith Wagener scale

	MAP (mmHg)		ECG changes		Fundus grade	
	<130	≥130	0	I/LVH	0–I	II/more
Untreated pats						
n	5/50	7/28	9/60	3/18	8/65	4/13
%	10 0	25 0	15 0	16 7	12 3	30 8
Treated pats						
n	6/42	5/41	0/23	11/59	2/36	9/47
%	14 3	12 2	0	18 6[b]	5 9	19 1
Total						
n	11/92	12/69	9/83	14/77	10/101	13/60
%	11 9	17 4	10 8	18 2	9 9	21 7[c]

One patient missed ECG recording [b] $p<0\ 02$ [c] $p<0\ 05$

patients with MAP ≥130 mmHg and MAP <130 mmHg The AB could not be detected in those with normal ECG whereas they were present in 18 6% of those with ECG abnormalities ($p<0\ 02$) Also in this group AB were more frequent in patients with fundus grade II or more Two males had grade III changes but no grade IV changes were observed No relationship was found between heart volume and the occurrence of AB accordingly these data are not shown

IgG ANA were not detected in the controls (Table IV) while they occurred in 5 1% of untreated

Table IV *Antinuclear antibodies (ANA) in relation to clinical data in 78 untreated and 83 treated patients with essential hypertension*

Abbreviations as in Table III

	MAP (mmHg)		ECG changes		Fundus grade		Total	Controls
	<130	≥130	0	I/LVH	0–I	II/more		
IgG ANA								
Untreated pats								
n	0/50	4/28	3/60	1/18	1/65	3/13	4/78	0/78
%	0	14 3	5 0	5 6	1 6	23 1[b]	5 1	0[d]
Treated pats								
n	3/42	2/41	0/23	5/59	1/36	4/47	5/83	
%	7 1	4 9	0	8 4	2 8	8 5	6 0	0[d]
Total								
n	3/92	6/69	3/83	6/77	2/101	7/60	9/161	0/78
%	3 3	8 7	3 6	7 8	2 0	11 7	5 6	0[d]
IgM ANA								
Untreated pats								
n	0/50	2/28	1/60	1/18	0/65	2/13	2/78	4/78
%	0	7 1	1 7	5 6	0	15 4[b]	2 6	5 1
Treated pats								
n	0/42	2/41	0/23	2/59	1/36	1/47	2/78	
%	0	4 9	0	3 4	2 8	2 1	2 6	5 1
Total								
n	0/92	4/69	1/83	3/77	1/101	3/60	4/161	4/78
%	0	5 8	1 2	3 9	1 0	5 0	2 5	5 1

$p<0\ 01$ [b] $p<0\ 005$ [c] $p<0\ 025$ [d] $p<0\ 05$

(p<0 05) and in 6 0% of treated patients (p<0 05) IgM ANA were found in 5 1% of the controls and in 2 6% of both hypertensive groups (n s) The relation between ANA and the clinical data is also shown in Table IV IgG ANA were not detected in untreated patients with MAP <130 mmHg, and were present in 14 3% of those with MAP ≥130 mmHg (p<0 01) Neither IgG-ANA nor IgM ANA were associated with ECG changes whereas IgG ANA were found in 23 1% of those with fundus grade II or more and in 1 6% of those with fundus grade 0–I (p<0 005) IgM ANA were also related to fundus grading In treated patients no association was found between ANA and MAP The frequency of IgG ANA was higher in patients with ECG changes and fundus grade II or more but the differences were not significant

A family history of hypertension was found in 23 5% of hypertensive males with AB as against 9 0% of males without (p<0 10) In female patients the frequencies were 16 7% and 13 0% respectively

DISCUSSION

The present investigation shows that titres of AB in untreated and treated patients with essential on of short known duration were higher in normotensive subjects with the same dis hy age and sex The overall frequency of the AB was as high in untreated as in treated patients but in neither group did it differ significantly from that in the controls The only exception to this was the frequency of IgG ANA (Table IV) In this connection it must be emphasized that the frequency of IgG ANA in the present control material agrees well with previous reports as we used a dilution of 1 20 or more (1 6 17 19) However the incidence of one or more AB was about twice as high in both groups of hypertensive males compared with the controls and the lack of a statistically significant difference could be due to the small numbers investigated as compared with population studies, and possibly also to the inclusion of borderline hypertensives

The cause and role of AB in essential hypertension are not known The incidence of some AB in normal persons has been demonstrated to be age related (1 17 19) This cannot account for the present findings as mean age was similar in patients with and without AB (Table II) Moreover untreated patients were about a decade younger than treated and the frequency of AB was not higher in the latter group The similar frequency of AB in both untreated and treated patients suggests that the increased occurrence of AB in essential hypertension cannot be ascribed solely to drug induction Neither could the AB be due to autoimmune liver or infectious diseases as patients with these disorders were excluded from the present study

In the Busselton population study, Mathews et al (14) were able to demonstrate an association of AB with cardiovascular diseases In this study the AB were associated with ECG changes in treated patients (Table III) and the IgG ANA with BP in untreated patients and with fundus grading in both groups of hypertensive patients (Table IV) An association of ANA with fundus grading has also been demonstrated by Feltkamp et al (6) Our results may therefore be regarded as a confirmation of the findings in the Busselton study The lack of an association of BP with AB in the treated patients is not surprising as BP in this group had been reduced by means of drugs which does not necessarily imply a restoration of any possible vascular changes

In untreated patients the AB occurred independently of ECG changes (Tables III IV) This finding contrasts with that in treated patients but the frequency of the AB was still about twice that found in the controls Mathews et al suggested that in some cases AB might play a pathogenetic role in the development of hypertension and vascular complications Cellular fragments from natural cell turnover or cell death were thought to act as antigens and induce formation of AB directed against vascular tissue with a subsequent increase in peripheral resistance and an increased BP (15) Whether the independence of AB from ECG changes in untreated patients supports that assumption remains unanswered at this stage of the study but if so the AB might be used as predictors of vascular lesions Moreover Folkow et al (7) have demonstrated an increase in the smooth muscle layer of the arteriolar vessels in patients with essential hypertension Such a process may possibly induce formation of SMA and could explain the increased incidence of SMA in the untreated patients in this study

It is also possible that the occurrence of AB is a manifestation of a genetic ability to react to vascular injuries with formation of AB Such a genetic ability could explain the equal frequency of AB in

untreated and treated patients and the independence from the duration of the hypertension In this context it is of interest that preliminary reports seem to indicate that essential hypertension is related to certain histocompatibility antigens (8 12) The observed higher frequency of a family history of hypertension among the males with AB could thus support this assumption and studies of the histocompatibility antigens in the present material are in progress

From this study it is not possible to evaluate whether AB play a secondary or a primary role in hypertensive vascular disease The patients and controls are therefore being followed in prospective studies However it can be concluded that the presence of AB in essential hypertension is not necessarily due to drug induction

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Antibodies against Double-Stranded DNA in Patients with Connective Tissue Diseases

Comparison between Crithidia Luciliae Kinetoplast Immunofluorescence Test and Farr Technique

Henrik Permin Poul Halberg and Erik Christiansen

From Immunological Laboratory University Clinic for Infectious Diseases Rigshospitalet Department of Medicine Section of Rheumatology Hvidovre Hospital Department of Clinical Chemistry Kommunehospitalet and Department of Medicine Y Bispebjerg Hospital Copenhagen Denmark

ABSTRACT Antibodies against double stranded (ds) DNA were demonstrated by an immunofluorescence technique using Crithidia luciliae kinetoplast as antigen, and by means of the Farr technique Both techniques were used simultaneously in 172 sera from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), juvenile rheumatoid arthritis (JRA), temporal arteritis (TA) and from healthy controls Comparable results were obtained with the two techniques SLE patients with active disease had higher titres of IgA antibodies than patients with inactive disease Of the patients with RA and JRA, 10% had significant titres of dsDNA antibodies Patients with TA and normal controls had either no dsDNA antibodies in their sera or very low titres without complement fixing properties

Autoantibodies against nuclear constituents may be of diagnostic significance in patients with various connective tissue diseases Antibodies against double stranded (ds) DNA are frequently found in sera from patients with systemic lupus erythematosus (SLE) but rarely in patients with other connective tissue diseases Several techniques are available for demonstrating dsDNA antibodies e g complement fixation hemagglutination counterimmunoelectrophoresis radioimmunoassay and immunofluorescence (2 3 7 8 10 13 15 16 23)

Recently Aarden et al (1) described a method in which Crithidia luciliae a homoflagellate is used as an antigenic substrate This organism besides a nucleus also contains a 0 7 μ kinetoplast in which DNA is concentrated in a large network The DNA is double stranded arranged in close circles interlocked by bonds and consequently very stable

In this study sera from patients with connective tissue diseases and controls were examined for the occurrence of nuclear antibodies by means of the Crithidia test the Farr technique and the classical ANF test

PATIENTS AND METHODS

Forty two patients had SLE according to the American Rheumatism Association criteria (9) Disease activity was estimated according to Schur and Sandson (16) The female/male ratio was 36/6 and the average age was 48 years (range 23–79) Nineteen patients had active disease and 7 had lupus nephritis as verified by renal biopsy

Forty patients 30 women and 10 men had classical or definite rheumatoid arthritis (RA) according to the criteria of the American Rheumatism Association (14) The average age was 58 years (range 36–88)

Twenty patients had juvenile rheumatoid arthritis (JRA) based on criteria of Ansell and Bywaters (4) Fourteen were girls and 6 boys The average age was 11 years (range 4–16)

Fifteen patients 10 women and 5 men had temporal arteritis (TA) verified by biopsy The average age was 74 years (range 63–84)

Fifty five sera were drawn from normal healthy blood donors 44 men and 13 women The average age was 40 years (range 20–64)

Serological studies

Crithidia luciliae cultures (A ı ı *Culture Collection USA)* w

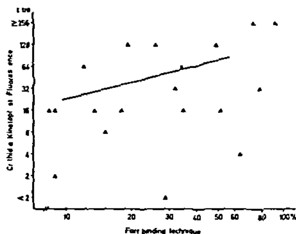


Fig 1 Correlation between serum DNA antibodies of patients with SLE assayed by Crithidia kinetoplast fluorescence and Farr binding techniques ▲=patients with clinically active disease ●=patients with clinically inactive disease

room temperature The organisms were washed four times with phosphate buffered saline (PBS) pH 7 2 A suspension was prepared in distilled water containing 40 mill organisms/ml Drops of 10 μl were applied to glass slides air-dried and fixed in 96% cold ethanol for 10 min The slides were frozen and stored at −25 C

The slides were incubated with sera in a moist chamber for 30 min followed by washing and ...sion in PBS twice for 10 min Conjugates were ap... and the slides were again incubated for 30 min ...owed by a second set of washings Cover glasses were finally mounted with a mixture of glycerol and PBS (1 2) and the slides were examined within a few hours Complement fixing properties were tested as described previously (22) All sera were examined in dilutions 1 2 1 4 1 8 and 1 16 Twofold titration of the positive sera was performed using PBS as diluent. Titres of ≥16 were considered abnormal for IgA IgM and IgG antibodies and titres of ≥2 for IgD and IgE antibodies and complement fixing properties

Conjugates

Fluorescein isothiocyanate (FITC) labelled rabbit IgG specific for human α γ and μ chains and βlc component of human C3 were purchased from Dakopatts Copenhagen Rabbit antisera specific for human δ and ε chains were obtained from Behringwerke West Germany The isolated IgG fractions were labelled with FITC (21)

The anti immunoglobulin conjugates were tested for specificity by means of IgA IgM IgG IgD and IgE monoclonal bone marrow specimens from patients with multiple myeloma and macroglobulinaemia (21) All conjugates showed monospecific reactions in crossed immunoelectrophoresis The fluorescein/protein ratios as estimated by OD 495/280 nm were 0 5–0 7 The sections were examined in a Leitz Orthoplan fluorescence microscope equipped for incident light illumination using an Osram HBO 200 mercury lamp as light source

The Farr assay was performed as described by Pincus et al (13) except that the precipitate formed by addition of ammoniumsulphate was isolated before measuring the radioactivity (8) The results were expressed as the radioactivity of the precipitate divided by the amount of radioactivity added Values of less than 0 20 were considered normal 0 20–0 30 questionably elevated and more than 0 30 definitely elevated (8) ^{14}C DNA was supplied by The Radiochemical Centre Amersham

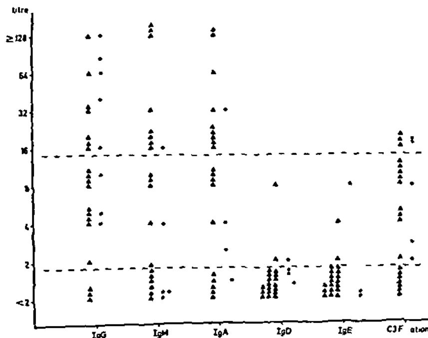


Fig 2 Distribution of serum immunoglobulins and complement C3 fixation DNA antibodies in patients with SLE estimated by Crithidia kinetoplast fluorescence technique Symbols as in Fig 1

Table I *Incidence of immunoglobulins and complement C3 fixation DNA antibodies (%) estimated by Crithidia kinetoplast fluorescence technique*

Subjects	IgG titres ≥2	IgG titres ≥16	IgM titres ≥2	IgM titres ≥16	IgA titres ≥2	IgA titres ≥16	IgD titres ≥2	IgE titres ≥2	C3 fixation titres ≥2	C3 fixation titres ≥16
SLE (n=42)	83	48	60	33	69	26	14	10	69	17
RA (n=40)	58	8	38	8	40	3	3	3	18	
JRA (n=20)	70	5	70		25	10	3	0	20	
TA (n–15)	27		33		27		6	0	0	
Normals (n 55)	24		11		13		2	0	0	

Sera were investigated for the occurrence and titres of IgG and complement C3 fixing granulocyte specific (GS) and organ nonspecific (ON) ANF Rat liver cryostat sections and smears of isolated and washed human leucocytes served as nuclear substrates (20 21 22) Sera were screened 1 16 for IgG ANF and undiluted for complement fixing properties

LE cell tests were carried out by the method of Hammer (12) Rheumatoid factors were demonstrated by the F II latex fixation slide test (Behringwerke) Titres of ≥32 were considered abnormal

Complement components C3 and C4 were studied by an immune diffusion technique (5)

Statistics

The statistical significance of the data was evaluated by the Mann Whitney rank sum test Fisher s four fold table test and linear correlation (11) Significance level 5%

RESULTS

dsDNA antibodies in titres of 16 or more as estimated by the Crithidia immunofluorescence technique were found in 69% of 42 sera from patients with SLE (Fig 1) Titres of ≥64 were found in 26% of the sera DNA antibodies were found by the Farr technique (20% binding or more) in 40% of the same sera and 26% of the sera revealed 30% binding or more A statistically significant correlation (r=0 48 p<0 01) was found when the highest Crithidia antibody titres (IgG IgM or IgA respectively) were plotted against the DNA binding score using Farr technique (Fig 1)

dsDNA antibody classes as demonstrated by the Crithidia technique are shown in Fig 2 and Table I Abnormal titres of IgG antibodies were present most frequently while IgD and IgE antibodies in low titres were found in only a few sera

The median IgA titre was 8 (range 0–256) for SLE patients with active disease and 2 (range 0–16) for patients with inactive disease (Fig 2) the difference being statistically significant (p<0 05) Titres of other immunoglobulin classes estimated by the Crithidia technique as well as DNA binding capacity were found to be unrelated to disease activity The complement fixing titres were unrelated to antibody titres and classes and to disease activity

IgG ON ANF was found in all SLE sera in titres of 1 32 or more three patients had GS ANF as well Complement fixing properties were found in all sera except one Twenty nine patients had positive LE cell tests and 16 had positive latex tests The complement fixing ANF titres were higher in SLE patients with than without nephritis (p<0 05)

Subnormal concentrations of complement C3 and C4 were found in 4 and 12 SLE patients respectively No correlations were found when comparing disease activity presence or absence of nephritis positive LE cell tests positive latex tests IgG ANF titres and concentrations of complement components

Four sera from patients with RA showed kinetoplast fluorescence in titres of ≥16 the highest titre being 32 (Table I) The Farr assay showed more than 20% binding in 5 sera (maximum 25%) Thirty five (88%) of the 40 RA patients had a positive ANF most titres being low Nineteen were GS ANF and 14 fixed complement in low titres Three patients had positive LE cell tests and 31 had positive latex tests No correlations were found when comparing DNA antibodies IgG ANF latex tests and complement components

Two JRA patients had Crithidia titres of 16 The Farr technique showed less than 20% binding in all patients Thirteen patients had positive IgG ANF 3 were GS ANF and 6 fixed complement in very low titres None had positive LE cell tests or latex tests No correlations were found when comparing DNA antibodies ANF and

All the patients with TA had Crithidia titres of less than 16 and less than 20% DNA binding by the Farr technique Four patients had IgG ANF in titres of 16 without complement fixing properties No patients had positive LE cell tests one patient had a positive latex test

Ten of the control sera showed low Crithidia titres (<16) One serum showed a DNA binding of 21% Three sera revealed abnormal ON ANF all in low titres and without complement fixing properties Three sera showed positive latex tests

DISCUSSION

dsDNA antibodies have been repeatedly demonstrated in sera of many patients with SLE (1 7 8 13, 15–18 23) The incidence varies with the source of dsDNA antibody avidity and the sensitivity of serological techniques (2 3) The indirect immunofluorescence technique performed on Crithidia luciliae and the Farr technique for determination of DNA antibodies both detect the primary interaction of DNA with antibody Therefore it is not surprising, that a correlation between titres of kinetoplast fluorescence and DNA binding capacity could be found in this study as well as in other reports (1 17) However quite a number of considerable discrepancies between the results of the two techniques e seen in all studies The explanation may be that antibodies against DNA rather than being a single homogeneous antibody constitute an entire family of antibodies which react with different antigenic sites on the DNA molecules (2 16)

SLE patients with active disease had higher titres of IgA DNA antibodies but disease activity could not be correlated to other immunoglobulin classes of DNA antibodies or to the presence of positive LE cell tests or increased DNA binding as previously reported (8 16)

ANF from SLE patients with renal disease fixed complement in higher titres than ANF from patients without renal involvement (19)

Up to 10% of the patients with RA and JRA had abnormal anti DNA titres and DNA binding capacity as described by others (6 16)

Low titres of Crithidia DNA antibodies (none of them complement fixing) were found in sera from patients with TA and in normal sera whereas the DNA binding was normal in these sera

Differentiation between staining of kinetoplast and nucleus of Crithidia luciliae generally gave no problems Some of the sera showed a higher titre of kinetoplast staining, than observed by ANF technique This phenomenon which has been observed in other anti DNA assays (15), may be due to the high concentration of DNA in the kinetoplast

Low titred anti DNA and ANF may not be pathological phenomena (20) In this study anti DNA in normal control sera never possessed complement fixing properties, which may be analogous to previous observations that low titred ANF occasionally found in normal sera are never complement fixing (20) Anti DNA may be a normal serum constituent serving a physiological purpose

In conclusion the kinetoplast fluorescence test for demonstrating dsDNA has the advantages of being cheap and easy to perform it allows the determination of immunoglobulin classes and the study of complement fixation

ACKNOWLEDGEMENTS

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The Effect of Hemodialysis on Neutrophil Chemotactic Responsiveness

Bengt Bjorksten [1] S Michael Mauer Elaine L Mills and Paul G Quie

From the Departments of Pediatrics University of Minnesota Medical School Mayo Minneapolis Minnesota USA

ABSTRACT After several hemodialysis treatments, neutrophil chemotactic responsiveness is commonly depressed The median chemotactic index of 34 patients was 21, compared with 47 for 21 controls tested simultaneously The depressed chemotactic responsiveness was not restored to normal when leukocytes were washed and resuspended in normal plasma neither did plasma from patients with depressed chemotaxis affect control neutrophils

Recently we demonstrated that severely depressed neutrophil chemotactic responsiveness may follow repeated hemodialysis treatment (5) and it was suggested that this phenomenon could in part explain the increased susceptibility to serious bacterial infections noted in uremic patients (3 9)

We have extended the studies of neutrophil function in patients on hemodialysis to a larger group and plasma factors and leukocytes from patients have been studied separately in an effort to explain the depressed chemotactic responsiveness We found that this responsiveness was not restored when the cells were resuspended in control plasma and that plasma from patients with depressed chemotaxis did not affect control neutrophils These results indicate that hemodialysis induced defective chemotaxis is not caused by lack of a necessary plasma factor or by the presence of a plasma factor that inhibits chemotaxis

[1] *Present address* Department of Pediatrics University of Umeå S 901 85 Umeå Sweden

STUDY POPULATION AND METHODS

Patients and controls All 34 patients were in renal failure for at least 2 months and had received at least 8 hemodialyses at the University of Minnesota Hospitals Renal failure was defined as a blood urea nitrogen higher than 40 mg/100 ml and a serum creatinine higher than 2 0 mg/100 ml Forty seven healthy adult volunteers served as controls Pooled human serum (PHS) was obtained from three healthy volunteers The serum was frozen within two hours of drawing and stored at −20°C prior to use Samples were only frozen and thawed over The study was approved by the Committee of the Use of Human Subjects in Research at the University of Minnesota

Chemotaxis The method has been described in detail elsewhere (7) Briefly leukocytes were separated by gravity sedimentation counted and diluted with Hank s BSS containing 30 mM HEPES (pH 7 4) to a final concentration of $2–4\times10^6$ polymorphonuclear leukocytes (PMN) per ml Cells from 0 4 cm^3 of this dilution were deposited onto a 5 micron millipore filter The filters were placed in a modified Boyden chamber with the supernatant of an overnight culture of E coli or zymosan activated serum (7) as cytotaxins After 3 hours of incubation the chemotaxis filters were removed and stained with hematoxylin

The number of PMN that had migrated completely through the filter in ten random high power fields (using a 5×5 mm reticule) were counted and divided by the number of cells ($\times10^6$) delivered to the starting side of the filter this giving the chemotactic index (CI)

To study the effect of plasma and leukocytes from the patients separately leukocytes from patients or controls were washed twice in Hank s BSS and then resuspended in plasma or PHS to a final concentration of $2–4\times10^6$ PMN/ml The plasma samples were either tested within six hours of drawing the blood or stored at −20°C prior to use

Hemodialysis technique Patients were treated with parallel flow cuprophane membrane dialyzers of the Lindea series (Gambro Lund Sweden) A Milton Ray

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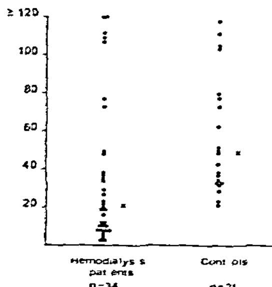


Fig 1 Chemotactic indices of patients undergoing repeated hemodialysis and healthy controls (individual and median values).

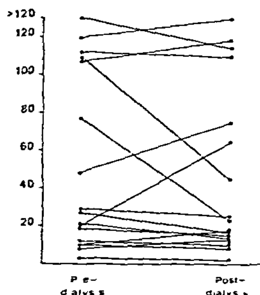


Fig 2 Chemotactic index of neutrophils drawn prior to and after 4 hours hemodialysis from 17 patients undergoing repeated hemodialysis

dialysis machine was used for mixing of the concentrate with deionized water and for delivery of the dialysate to the dialyzer Ascorbic acid, 2–4 g, was added to the dialysate concentrate in order to prevent the methemoglobinemia caused by chloramines present in the water (8)

Statistical methods Student's *t*-test for paired observations was employed.

RESULTS

The median of CIs was lower in patients on hemodialysis than in the healthy controls (Fig. 1) However as shown in the figure, there was a great overlap between the two groups and several patients had CIs well within the normal range

An individual hemodialysis treatment usually did not influence chemotactic responsiveness. In fact, there was no significant difference between neutrophils from the same patients prior to and after 4 hours hemodialysis (Fig. 2)

Plasma drawn from patients with depressed chemotactic responsiveness before or after four hours of hemodialysis did not significantly depress chemotactic responsiveness of neutrophils from controls (Table I) Plasma samples drawn prior to and after 4 hours of dialysis did not differ significantly from pooled serum with respect to capacity to generate chemotactic factors when incubated with zymosan (Table II) In three experiments we compared the chemotactic responsiveness of patient leukocytes incubated in autologous plasma and in PHS PHS did not restore the depressed chemotactic responsiveness

The possibility that the dialyzer membranes could either activate plasma inhibitors to chemotaxis or remove factors necessary for a normal chemotactic responsiveness was further studied by passing normal PHS and serum chelated with 10 mM EDTA, or 10 mM Mg^{++} EGTA through the dialyzer or by incubating these sera with dialysis

Table I *Effect of plasma from patients with defective chemotaxis on the chemotactic responsiveness of normal polymorphonuclear leukocytes (PMN)*

Results are shown as mean chemotactic index (CI) of 11 experiments in which cells were suspended in normal pooled human serum (PHS) or plasma from patients prior to or after 4 hours dialysis

PMN suspended in	CI (mean ± S E M)
PHS	57 ± 14.5
Predialysis plasma	50 ± 9.9
Postdialysis plasma	42 ± 10.1

Table II *Generation of cytotaxins in plasma from patients with abnormal chemotactic activity prior to and after 4 hours of dialysis*

Results are expressed as mean chemotactic index (CI) of normal polymorphonuclear leukocytes in 8 experiments using 10% plasma incubated with zymosan as cytotaxin

Source of cytotaxin	CI (mean±S E M)
Pooled human serum	27± 6 8
Predialysis plasma	33±10 3
Postdialysis plasma	29± 4 9

membranes in vitro Normal neutrophils incubated in these sera did not differ from cells incubated in control serum with regard to chemotactic responsiveness

DISCUSSION

In an earlier study we demonstrated that patients with chronic renal failure not on hemodialysis had mildly depressed neutrophil chemotactic responsiveness (5) thus confirming the work of Baum et al (1) Further this work demonstrated that chemotaxis was markedly depressed after eight or more hemodialysis treatments and that impaired chemotactic responsiveness could develop after several dialyses in uremic patients whose initial CIs were normal (5) Salant et al (11) reported that patients had abnormal CIs i e less than 20 We creased neutrophil chemotaxis of a severity equal to or more severe than that of patients on regular hemodialysis In this study we confirm on a larger number of patients our previous findings However as shown in Fig 1 only about 50% of our patients had abnormal CIs i e less than 20 We cannot explain the individual variations in our patients or the difference between the results of on the one hand Salant et al (11) and on the other of the present study and that of Baum et al (1)

No significant differences in CI were seen in our patients when we compared neutrophils obtained immediately before dialysis with those obtained after 4 hours of hemodialysis treatment This confirms previously published findings (1) and supports our earlier suggestion that the hemodialysis associated depression in CI develops slowly and requires several hemodialysis treatments to become manifest (5)

Recently Henderson et al (6) reported that dialysis membranes severely impaired random mobility of PMN and they suggested a serum factor to be responsible for this However we found that plasma from dialysis patients did not significantly had no effect on the responsiveness of normal neutrophils to a chemotactic stimulus and that plasma from dialysis patients did not significantly inhibit chemotaxis of normal PMN Thus our findings do not support the presence of inhibitory factors to chemotaxis in dialysis plasma Also we were unable to restore chemotactic responsiveness of patient PMN by incubating them in normal serum This indicates that the low CI in the patients was caused by a cellular defect and not by lack of a humoral factor

A direct effect of the dialysis membrane on the PMN is possible since granulocytes obtained by adherence to nylon fibers show morphological and biochemical evidence of degranulation (10) However since we found depressed chemotactic responsiveness in samples drawn up to 72 hours after the previous hemodialysis i e in PMN that had not been present in the circulation when the patient was dialyzed a direct effect of dialysis membranes on the PMN in the circulation is unlikely

During hemodialysis there is an initial neutropenia due to sequestration of peripheral neutrophils in the lung and a subsequent rebound neutrophilia due to release of these sequestered neutrophils as well as release of neutrophils from the bone marrow (2) Repeated hemodialysis may reduce bone marrow neutrophil reserves and induce morphological changes in granulocytopoietic bone marrow cells including cytoplasmatic vacuolization and lysis of chromatin (4) Apparently repeated hemodialysis interferes with normal granulocyte maturation in the bone marrow If the depressed chemotactic responsiveness of neutrophils is a consequence of a disturbed maturation this would explain why several hemodialyses were required to induce a depressed chemotactic responsiveness and why the chemotactic index did not vary significantly with an individual hemodialysis Our studies would support the idea of an effect on bone marrow cells rather than the presence of a serum factor affecting circulating neutrophils

The studies show that repeated hemodialysis induces changes in neutrophil locomotion Further studies are needed to precisely characterize the mechanisms for the changes in neutrophil function and to define the clinical consequences

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Cerebrospinal Fluid Lysozyme in Bacterial and Viral Meningitis

Matti Klockars Sakari Reitamo Teddy Weber and Yrjo Kerttula

From Fourth Department of Medicine University of Helsinki and Aurora Municipal Hospital Helsinki Finland

ABSTRACT The concentration of lysozyme (LZM) in cerebrospinal fluid was determined in 25 patients with bacterial meningitis, in 18 patients with viral meningitis and in 25 control patients who had other febrile illnesses The concentration of LZM was less than 1 5 μg/ml in all control patients, and slightly to markedly raised in 10 patients with viral meningitis and in 11 out of 13 patients with untreated bacterial meningitis The concentration of LZM was significantly different in the viral and bacterial meningitis paitents ($p<0\ 001$) Most raised concentrations of cerebrospinal fluid LZM persisted for at least one week after the start of antibiotic treatment The concentrations of LZM correlated well with concentrations of lactic dehydrogenase These results show that the determination of cerebrospinal fluid LZM is a useful tool in the differential diagnosis of menigitis, particularly when the prehospital treatment with antibiotics may be responsible for a diagnostically misleading negative bacterial culture of the cerebrospinal fluid and altered cerebrospinal fluid cytology

In the diagnosis of bacterial meningitis conventional analyses of cerebrospinal fluid i e differential cell counts determinations of protein concentration Gram stains and cultures do not always give reliable results The search for additional diagnostic criteria with which to assess inflammatory disorders of the central nervous system has prompted the study of a variety of enzyme activities in the cerebrospinal fluid The concentration of lactic dehydrogenase (LDH) including certain of its isoenzymes in cerebrospinal fluid has been observed to be raised in bacterial meningitis when compared with the concentration in meningitis of viral etiology (1 9) Concentrations of cerebrospinal fluid lysozyme (LZM) have been shown to be raised in bacterial (including tuberculous) and fungal meningitis (4 11 12)

This study was undertaken to assess the possible significance of determinations of cerebrospinal fluid LZM in the differential diagnosis of viral and bacterial meningitis We were particularly interested to see whether the determination of cerebrospinal fluid LZM would be of value in the diagnosis of bacterial meningitis in patients who had received antibiotics

STUDY POPULATION AND METHODS

Patients LZM concentration in cerebrospinal fluid was studied in 25 patients with bacterial meningitis Samples of cerebrospinal fluid were taken from 13 of them before the start of antibiotic treatment Two or more samples from 9 of these 13 patients were analyzed during the course of treatment In these 13 patients the causative microorganisms were *Neisseria meningitidis* group A in 8 patients *Diplococcus pneumoniae* in 2 *N meningitidis* group C in 1 patient a coliform strain in 1 and unknown in 1 patient In the remaining 12 patients with bacterial meningitis the cerebrospinal fluid specimen(s) were studied 2–18 days after the start of antibiotic treatment In these 12 patients the responsible bacteria were *N meningitidis* group A in 9 patients *Diplococcus pneumoniae* in 2 and *Listeria monocytogenes* in 1 patient.

We also studied 18 patients whose clinical and cerebrospinal fluid findings were consistent with serous meningitis and who were therefore included in the viral meningitis group The etiologic agents were mumps in 7 patients Coxsackie B 5 in 1 patient Coxsackie B 3 in 1 Echo 7 in 1 and unknown in 8 patients

The control group comprised 25 patients admitted to hospital for acute febrile illnesses All had had signs of meningeal irritation that warranted lumbar puncture but study of their cerebrospinal fluid revealed no cellular or

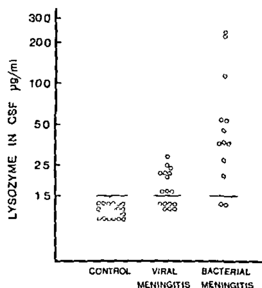


Fig 1 Concentration of LZM in the cerebrospinal fluid of 25 control patients 18 patients with viral and 13 with bacterial meningitis

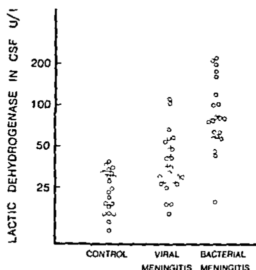


Fig 2 Concentration of LDH in the cerebrospinal fluid of 23 control patients 34 patients with viral and 23 with bacterial meningitis

protein abnormalities and no evidence of a specific viral or bacterial meningeal inflammation

Bacterial cultures differential leucocyte counts and determinations of glucose and protein concentrations were performed on all cerebrospinal fluid specimens

LDH activity in cerebrospinal fluid was determined in another series of patients 23 controls 34 patients with viral meningitis and 23 with bacterial meningitis Fig 4 shows the number of patients with meningitis in whom enzyme values were determined

LDH was assayed as recommended by the Scandinavian Society for Clinical Chemistry and Clinical Physiology (13) Concentrations of LZM were determined by the lysoplate method described by Osserman and Lawlor (10) in which purified human LZM is the standard LZM concentrations as low as 1 5 µg/ml (which corresponds to 4 3 µg/ml when the standard used is hen-egg white LZM) could be measured by this method

RESULTS

Fig 1 shows the concentrations of LZM detected in the cerebrospinal fluid of control patients and of patients with viral and bacterial meningitis In all control patients this concentration was less than 1 5 µg/ml Of the 18 patients with viral meningitis 8 had concentrations of less than 1 5 µg/ml and 10 within the range 1 5–2 9 µg/ml (mean±S D 1 6±0 5) Among the patients with bacterial meningitis only 2 out of 13 had LZM levels below 1 5 µg/ml whereas the concentrations were considerably raised in 11 2 1–24 0 µg/ml (mean±S D 7 1±7 6) The difference between the viral and bacterial meningitis groups was statistically significant ($p<0\ 001$)

Fig 2 shows the concentrations of LDH detected in the cerebrospinal fluid of control patients and patients with viral and bacterial meningitis The mean concentrations (±S D) were 25 5±8 2 39 7±22 8 and 99 6±57 4 U/l respectively The *difference between control patients and patients* with viral meningitis as well as between patients with viral and bacterial meningitis were statistically significant ($p<0\ 001$)

Fig 3 shows the changes in the concentration of

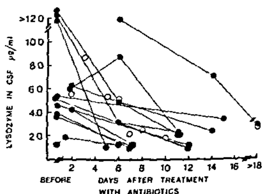


Fig 3 Concentrations of cerebrospinal fluid LZM during treatment with antibiotics Serial samples from cerebrospinal fluid were taken from 13 patients (●—●) and from 8 patients once after the start of treatment (○) □=Values *below the lowest detectable concentration* which include those for all 25 control patients

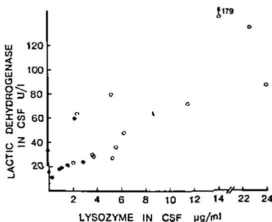


Fig 4 Correlation between concentrations of LDH and LZM in cerebrospinal fluid of patients with viral (●) and bacterial (O) meningitis The cerebrospinal fluid specimens analyzed were taken both before and after the start of treatment

cerebrospinal fluid LZM during treatment with antibiotics Nine out of 14 patients still had raised levels of LZM 2–7 days after the start of treatment and in 4 out of 21 patients they were still raised after 14–21 days of treatment

Fig 4 shows that in 20 patients with bacterial meningitis the concentration of LDH correlated with that of LZM ($r=0\ 6921$) but not in patients with viral meningitis ($r=0\ 2576$)

In patients with bacterial meningitis the concentration of LZM in cerebrospinal fluid did not correlate either with the concentration of protein or with total leucocyte or granulocyte counts Nor was there any relationship between the content of LZM in cerebrospinal fluid and the causative microorganism or the outcome of illness

DISCUSSION

Under normal conditions the concentration of LZM in cerebrospinal fluid is slight and bears no relationship to the concentration in serum (5 11 12) It has been postulated that meningeal infections alter the permeability of the blood–brain barrier with the result that in cerebrospinal fluid changes in enzyme activities usually parallel changes in the protein concentration (14)

In bacterial meningitis the isoenzymic pattern of LDH in cerebrospinal fluid suggests that this enzyme does not originate either in serum or in the central nervous system but is derived from cerebrospinal granulocytes (1) That under normal conditions almost 80% of plasma LZM is derived from the turnover of neutrophilic granulocytes (2) that LZM is present in high concentrations in other pyogenic exudates (3 7) and that in this study LZM levels correlated significantly with LDH levels in cerebrospinal fluid all point to the granulocytic origin of the LZM in cerebrospinal fluid No correlation has been observed between the concentration of LDH and the granulocyte count in cerebrospinal fluid neither did the LZM levels correlate with granulocyte counts The probable explanation of this is that the movement of cells within the cerebrospinal fluid might be inhibited by fibrin deposits and by cerebrospinal fluid viscosity (1) The raised concentration of cerebrospinal fluid LZM during later stages of the disease might be due to the release of LZM from mononuclear phagocytes

Diagnostically important is the persistence of raised levels of LZM for several days after the start of specific antibiotic therapy The administration of antibiotics before admission to hospital not uncommonly accounts for a negative bacterial culture and a masked chemical and cytological composition of cerebrospinal fluid which in turn might be diagnostically misleading The prolonged rise in the concentration of cerebrospinal fluid LZM in bacterial meningitis contrasts with observations that concentrations of cerebrospinal fluid LDH tend to return to normal within 5–7 days (1)

Our results show that in the differential diagnosis of infections of the central nervous system the determination of cerebrospinal fluid LZM is a useful ancillary procedure Available methods permit the inexpensive and reliable assay of this LZM activity (6 8 10)

ACKNOWLEDGEMENTS

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Factors Influencing the Occurrence of "on-off" Symptoms during Long-Term Treatment with L-dopa

Ann Kathrine Granérus

From the Department of Geriatrics and Long Term Care Medicine Vasa Hospital University of Gothenburg Gothenburg Sweden

ABSTRACT On off" symptoms were found to have developed in 43 of 85 parkinsonian patients who had been treated with L-dopa for five years or more, the risk of such symptoms apparently being greater the younger the patient had been at the debut of the disease and at the start of treatment The dopa dose had been higher throughout the treatment in the patients developing ' on off" symptoms than in those maintaining an even effect, and, furthermore, the initial improvement had been more marked and dyskinesia had appeared earlier and in a higher frequency The clinical observations in the present studies seemed to be related to some pharmacological findings where different degrees of the nigrostriatal neuron degeneration and the efficacy of the remaining neurons could be of importance for a varying therapeutic response It also seemed possible that a higher dopa dose could evoke "on-off" symptoms more easily than a lower one in thereto predisposed individuals As the patients maintaining an even symptomatology during long term treatment with L-dopa were older, and in particular as they had dementia in a higher frequency than those developing on off" symptoms the possibility of a more wide spread neuron damage, influencing the clinical manifestation in these patients, had to be taken into consideration

The introduction of L dopa therapy implied the chance of a considerable improvement for many parkinsonian patients The chronic nature of Parkinson s syndrome meant that only after long term treatment with L dopa one could assess whether the course of the disease had been affected Would the early very good results which are often obtained last or would the disease continue to progress in spite of L-dopa treatment? Furthermore would new problems turn up as treatment proceeded?

With the problems mentioned above as a back ground it was decided to perform a follow up study of a group of 134 patients with Parkinson s syndrome in whom L dopa treatment had been instituted between May 1968 and June 1970 at Vasa Hospital and in the Neurologic Clinic at Sahlgren s Hospital in Gothenburg Sweden and in whom the clinical results had been analysed at a first follow up in Dec 1970 (18) The follow up to be presented here was performed in Dec 1975 when some patients could have been treated with L-dopa for 91 months as a maximum Eighty five of the patients were found to have been treated with L-dopa alone or combined with a peripheral dopa decarboxylase inhibitor for five years or more and in the present report special attention will be paid to these long term results

STUDY POPULATION AND METHODS

It was possible to obtain information on all the original 134 parkinsonian patients when the present follow up was performed although quite a few of them resided at that time in other places than Gothenburg It was found that 47 of them had died and a death certificate was obtained in all these cases Eighty three of the patients had been examined repeatedly by the author until death or the time of the follow up To obtain the requisite information concerning the remaining patients contact was established with the patient and/or doctors and nurses and case records were collected from hospitals and nursing homes

Detailed information was compiled about the therapy with L-dopa alone or combined with a peripheral decarboxylase inhibitor The date when such therapy was instituted or withdrawn was noted and the reason for the with-drawal The occurrence of side-effects especially those regarded as of central dopaminergic nature and whether such side effects had changed with changes in therapy was noted Doses of L-dopa alone or with inhibitor were recorded i e the first optimal do se

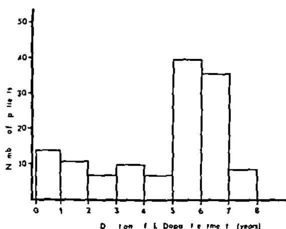


Fig 1 Duration of treatment with L-dopa alone or with inhibitor in 134 parkinsonian patients

in Dec 1970 when such a drug had been withdrawn and the dose in Dec 1975 or at death The degree of handicap was recorded according to a method described earlier (18) in which the handicap is expressed as a number of ADL (Activity of daily living) disability scores a minimum 0 score implying total freeness from parkinsonian symptoms and a maximum of 4 ADL scores implying such a serious handicap that the patient needed nursing concerning all the activities of daily living The ADL disability score was registered before the start of L-dopa treatment in Dec 1970 at a change from dopa alone to dopa combined with inhibitor at withdrawal of dopa therapy and in Dec 1975 or at death A previously performed stereotaxic operation was noted as was therapy with other drugs hich could possibly have influenced the result of the iparkinsonian therapy A note was also made of ether the patient had symptoms of dementia (obvious changes in memory function occurrence of mental confusion insufficient orientation concerning time place and personalia) before the start of L-dopa treatment in Dec 1975 or at the time of death

The presence of an on-off phenomenon (5 14 32) was especially looked for and attempts were made to find out when such symptoms began to appear The definition of an "on-off" phenomenon was a clear variation in the parkinsonian symptomatology during the day connected with the intake of L dopa where periods of good mobility appearing and ceasing quickly alternated with periods of markedly increased immobility Such periods of good mobility often become shorter as time passes and can even fail to occur after certain L-dopa doses The mobile periods are often but not invariably connected with involuntary movements

Statistical methods

Standard methods were used for calculating the mean (M) the standard deviation (S D) and the standard error of the mean (S E) The hypothesis of no differences in means was tested with Student s t test or the Wilcoxon Mann Whitney test for ranking of unpaired measurements The hypothesis of no difference between paired observations in the same subjects was tested with Student s t test for paired observations The hypothesis of no difference in proportions between two groups was tested with the χ^2 test except for some cases where Fisher's exact test of significance (15) was used The correlation of the frequency of subsequent on-off symptoms to the age at the commencement of Parkinson s syndrome and to the age at the start of L-dopa treatment respectively was tested with Point Biserial correlation (21) Differences were considered significant for p values of 0 05 or less

RESULTS

General study population

The duration of treatment with L dopa alone or combined with inhibitor is presented in Fig 1 which also shows that 85 of the 134 patients had been treated with L-dopa for five years or more nine of them for seven years or more

Forty seven of the original 134 patients, 24 women and 23 men had died since the start of the study (Table I) Autopsy was performed in 22 of them and all but two of the others died in hospitals or nursing homes where they were well known and the cause of death was clear One of two who died at home and in whom no autopsy was performed had stayed in a hospital a few months earlier and

Table I *Main cause of death in the deceased parkinsonian patients in the total series (n=134) and in the patients treated with L dopa for five years or more (n=85)*

	No of patients	
	Total	Treated for ≥5 y
Congestive heart failure	10	2
Myocardial infarction	4	1
Sudden death	1	–
Bronchopneumonia NUD	8	3
Bronchopneumonia caused by aspiration	4	–
Asphyxia	3	–
Cerebral vascular lesion	3	1
Ileus	2	–
Ischemic necrosis of intestine	1	–
Uremia	2	2
Sepsis caused by pyelonephritis	1	–
Intoxication (suicide?)	1	–
Anemia+splenomegalia NUD	1	–
Malignant disease in		
Breasts	2	–
Ovaries	1	–
Lungs	1	–
Stomach	1	–
Pancreas	1	–
Total	47	9

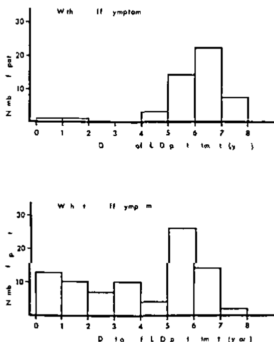


Fig 2 Duration of treatment with L dopa in patients with (n=48) and without (n=86) on off symptoms

was then attended by his house doctor until the time of death The other patient in whom autopsy was not performed had been found dead in his home during the night L dopa therapy was known to have produced postural hypotension in this patient

The main causes of death are shown in Table I Cardial and pulmonary diseases were the most frequent diagnoses In six patients aspiration leading to asphyxia or bronchopneumonia had caused death One patient who at autopsy was found to have atrophy of cerebellum and brain stem had died in an asphyxial picture probably caused by central respiratory deprivation Ileus had been the main cause of death in two patients both treated with anticholinergic drugs Six patients had died because of malignant diseases The malignant diagnosis was known before the start of L dopa treatment in two of these patients and two further patients had been treated with L dopa for less than a month several years before the malignant disease appeared In two cases the malignant disease was discovered after a rather long period of L dopa treatment i e in one patient with breast cancer and in one with cancer in the stomach after 14 and 20 months of treatment respectively Furthermore one patient had been treated with L-dopa for 14 months when severe anemia and splenomegaly were discovered the nature of which remained obscured

When the results were analyzed it was found that on off phenomena had appeared in many patients i e in 48 of the original 134 (Fig 2) The number of patients with on off symptoms increased with increasing duration of L dopa treatment In a few patients the on off phenomenon had appeared fairly soon after the start of treatment but in most cases after more than one year (Fig 3) in approximately 95% of the cases the on off phenomenon had started within the first five years of treatment Most patients had continued with L dopa treatment in spite of the on off symptoms and 43 patients 17 women and 26 men had been treated for five years or more (Fig 2)

It was further found that another 42 patients 23 women and 19 men had been treated with L dopa for five years or more without developing on off symptoms (Fig 2) The state of these patients was fairly constant during the day with no obvious variations in the parkinsonian symptomatology related to the dosage of L dopa

Because of this different symptomatology in different patients during long term treatment with L dopa it seemed reasonable to divide the patient material into two groups (Fig 2) one comprising those who had developed on off symptoms and the other exhibiting a more constant even symptomatology However some of the patients with an even symptomatology had received L dopa treatment for only a short time and might develop on off symptoms later if treatment continued The risk of on off symptoms occurring later in patients with an even symptomatology ought to be considerably smaller among those who had already been treated for five years or more (see above and Fig 3) Thus in the present report the results will

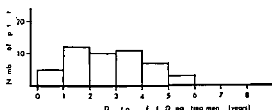


Fig 3 Duration of treatment with L-dopa at the beginning of on-off symptoms (n=48)

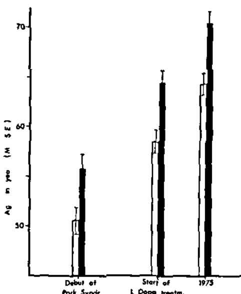


Fig 4 Age at the beginning of Parkinson s syndrome at the start of L dopa treatment and at the present follow up in 1975 (including the age at death in the nine deceased patients) in patients treated for five years or more □= On off group (n=43) ■= even group (n=42)

be compared between two groups of patients one comprising those who have developed on off symptoms called the on off group (n=43) and the other those with an even symptomatology called the even group (n=42) all having been treated with L-dopa alone or with inhibitor for five years or more In Dec 1975 all the patients in the on off group were alive while nine in the even group had died (Table I) It was decided to include the latter in the present follow up using findings shortly before the appearance of the conditions leading to death

Comparison between the on off and the even group

Age The mean age of the patients was significantly lower in the on off group than in the even group at the commencement of the disease (p <0 01), at the start of L dopa treatment (p< 0 001) and at the present follow up (p<0 001) (Fig 4) It was also found that the percentage of patients with subsequent on off symptoms was significantly higher the younger the patient had been at the commencement of Parkinson s syndrome (p<0 002) (Fig 5) and at the start of L dopa treatment (p<0 001) (Fig 6) The duration of the disease did not differ however between the on off and the even' group being approximately eight years in both (8 2±0 67 and 8 8±1 05 respectively) (M±S E) at the start of L dopa treatment and approximately 14 years (13 9±0 62 and 14 1±0 93, respectively) at the present follow up

L dopa dose The maintenance dose of L dopa was found to have been significantly higher in the on off and the even group being approximately eight years in both (8 2±0 67 and 8 8±1 05 respectively) (M±S E) at the start of L-dopa treatment and approximately 14 years (13 9±0 62 and dopa combined with inhibitor i e 26 patients in the on off group and 16 in the even group, the remaining patients being treated with L dopa alone

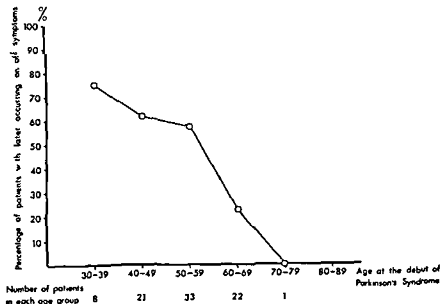


Fig 5 Percentage of patients with subsequent on-off symptoms among 85 patients treated with L-dopa for five years or more correlated to the age at the beginning of Parkinson s syndrome

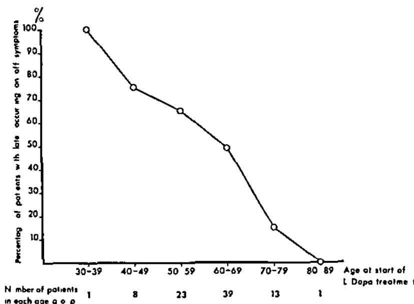


Fig 6 Percentage of patients with subsequent on-off' symptoms among 85 patients treated with L dopa for five years or more correlated to the age at the start of L-dopa treatment

The mean dopa doses were higher in the on off' group than in the even group for treatment with dopa alone as well as for dopa combined with carbidopa (Sinemet®) and dopa combined with benserazide (Madopark®) but the inter group differences were statistically significant only for the L dopa doses with Sinemet ($p<0$ 02) However resorption studies and clinical experience have shown that dopa combined with inhibitor usually corresponds to a 4–5 times higher dose of dopa alone (9 12 30 34) To be able to compare the maintenance doses of L-dopa among all the 85 patients in the present follow up one could as a fairly good approximation multiply the amount of L-dopa when given with inhibitor by a factor of four or five thus achieving a reasonable estimate of what the dopa dose would have been without inhibitor According to such calculations also at the present follow up the maintenance dose of L-dopa was significantly higher in the on off' than in the even group both when using a factor of four ($p<0$ 025) and of five ($p<0$ 005) (Fig 7)

The maintenance dose of L-dopa did not correlate statistically to the degree of improvement (expressed in terms of the number of improved ADL scores) in either of the groups although in the on off' group some of the patients with the greatest initial improvement had had the highest doses However in the even group the patients with the greatest initial improvement had had lower dopa doses than most of the patients with a smaller improvement

There was no significant correlation between the magnitude of the dose and the time when on off' symptoms began to appear although the patients with the earliest occurrence of on-off' symptoms had the highest and those with the latest occurrence the lowest maintenance dose

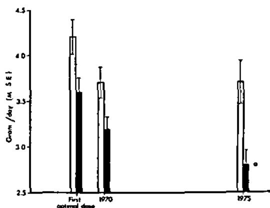


Fig 7 Maintenance dose of L dopa at the first optimal dose level at the previous follow up in 1970 and at the present follow up in 1975 (including the L-dopa dose shortly before death in the nine deceased patients) in the patients treated for five years or more *a*=Adjusted L-dopa doses including a fivefold multiplication of the amount of L-dopa when combined with inhibitor Symbols as in Fig 4

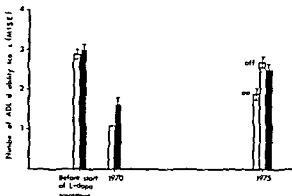


Fig 8 Number of ADL disability scores before the start of L-dopa treatment at the previous follow up in 1970 and at the present follow-up in 1975 (including the ADL scores shortly before death in the nine deceased patients) in the patients treated for five years or more Symbols as in Fig 4

Degree of disability At the start of L-dopa treatment there was no difference between the on-off and the even groups concerning the degree of disability caused by the parkinsonian symptoms (Fig 8) However after the initial period of treatment with L-dopa the on-off group had achieved a significantly lower number of ADL scores than the even group ($p<0.05$) (Fig 8) i e the initial improvement had been more pronounced in the on-off than in the even group

Owing to the different degrees of disability during the on and the off periods, it is difficult to compare the situation at the present follow up with a previous condition However at the present follow up both the on-off and the even group had deteriorated considerably compared with their state five years earlier but the number of ADL disability scores was still significantly lower than before the start of L-dopa treatment both in the on-off group although only for the on periods ($p<0.001$) and in the even group ($p<0.025$) (Fig 8)

Dyskinesia and muscular hypotonia Dyskinesia (involuntary movements) had occurred earlier during L-dopa treatment, and was found in a higher frequency in the on off than in the even group (Fig 9) In 91% of the patients in the on-off group, but in only 40% in the even group ($p<0.0005$) dyskinesia first appeared during the first year of L-dopa treatment By the present follow up all the patients in the on-off group had shown this side effect which was partly due to the symptomatology during the on periods but only two thirds in the even group ($p<0.00001$) Among the 14 patients in the even group who had never had dyskinesia seven had had a maintenance dose of L-dopa exceeding 4 g/day, one of them as much as 8 g/day for a couple of months

In 22 of the patients treated with L-dopa for five years or more an early occurrence of attacks with muscular hypotonia had been observed Of these 22 patients 15 (66%) had later developed on-off symptoms In the on-off group 35% of the patients had had an early occurrence of muscular hypotonia against 17% in the even group, but this difference was not statistically significant However an early occurrence of both muscular hypotonia and dyskinesia in one and the same pa-

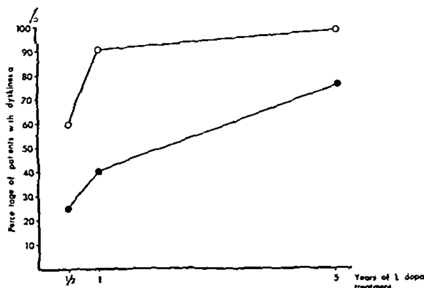


Fig 9 Percentage of patients with dyskinesia among patients treated for five years or more when the duration of L-dopa treatment was half a year one year and five years respectively
○="On-off" group ($n=43$)
●="even" group ($n=4_)$

tient was significantly more frequent ($p<0.025$) in patients who later developed an on off phenomenon than in those who maintained an even effect

Dementia At the start of the present study no patient in the on off group but nine (21%) in the even group had had symptoms of dementia At the follow up after treatment with L dopa for five years or more six patients (14%) in the on off group and 17 (40%) in the even group had symptoms of dementia The number of patients with dementia was significantly higher in the even than in the on off group both at the start of L dopa treatment ($p<0.001$) and at the time of the present follow up ($p<0.01$) At the present follow up patients with dementia had a significantly higher mean age ($p<0.01$) than those without dementia in the on-off group but the corresponding difference in the even group was not significant However in the two groups combined the patients with dementia were significantly older than those without dementia 71 8±1 30 versus 65 7±1 10 years ($p<0.001$) The duration of the disease on the other hand did not differ significantly between patients with and without dementia Concerning the effects of stereotaxic surgery it was found that dementia had developed in three of 15 operated patients in the on off group and in three of five operated patients in the even group However in the two groups combined symptoms of dementia at the present follow up were found in 30% of the operated patients and in 26% of the non operated patients a difference which is not significant

Involuntary movements had been observed in all the patients who had developed dementia in the on off group but in less than half (eight patients out of 17) of those with dementia in the even group

L dopa combined with inhibitor When this study was started in 1968 only L-dopa without a peripheral decarboxylase inhibitor was available Treatment with L-dopa in combination with inhibitor (Sinemet® Madopark®) was started in our clinics in 1973 but these drugs were not licenced in Sweden until May 1975 When the therapy was changed from dopa alone to dopa combined with inhibitor all the patients had been treated with L dopa for a long time 35–78 months The reason for changing therapy was unsatisfactory improvement of the parkinsonian symptoms often due to dose limiting side-effects and the existence of disturbing on off symptoms When dopa combined with inhibitor was given the dose was increased gradually and it usually took about one month to reach the maintenance dose The amount of dopa given was between 1/3 and as little as 1/20 although most often around 1/5 of the previous dose of dopa when given alone

Thirty nine patients in the on off group and 24 in the even group had treid dopa combined with inhibitor several had tried both of the drugs available No difference was found between these two drugs concerning either the effect on parkinsonian symptoms or side effects but a preference for one of the drugs could be seen in some patients At the time of the present follow up 26 patients in the on-off group and 16 in the even group were on this combined therapy the treatment having been withdrawn and replaced by dopa alone in the other patients

In eleven of the patients in the on off group the number of ADL disability scores had improved at least periodically during the day when the treatment was changed from dopa alone to dopa with inhibitor the improvement amounting to 1 ADL score in all 11 In 22 patients in the on off group the number of ADL disability scores did not change and the remaining six patients showed an impairment of the ADL function when treatment was changed from dopa alone to dopa combined with inhibitor An improved number of ADL disability scores did not always correspond to a more even effect in terms of on off symptoms Thus of the 11 patients with improved ADL function only eight had had a more even effect on the on off symptoms Furthermore ten of the 22 patients with an unchanged ADL function and three of the six with an impaired ADL function had had an initial improvement of the on off symptoms in most cases for one to three months treatment but after that the on off symptoms deteriorated often suddenly during a week or two and in some patients the on off symptomatology became more disturbing on dopa combined with inhibitor than when used alone

When the treatment was changed from dopa alone to dopa with inhibitor in the even group the number of ADL disability scores decreased in six patients the improvement amounting to 2 ADL scores in one patient and 1 ADL score in five patients The remaining 18 patients showed no change in the ADL disability score One very disabled patient did have an initial period with marked improvement but after about two months of dopa

with inhibitor the parkinsonian symptoms reappeared to the same extent as before although there was no change of the antiparkinsonian treatment

Around 50% (20 patients) in the on-off group and 25% (six patients) in the even group found the dyskinesia more disturbing during dopa with inhibitor than on dopa alone In one patient in the even gorup who had dyskinesia on the combined treatment this side effect had not developed with L-dopa alone even though the maintenance dose was rather high

Treatment with dopa alone was considered to have caused mental side-effects in five of the 39 patients in the on off group and in five of the 24 patients in the even group but the mental symptoms had disappeared when the dopa dose had been reduced When the treatment was changed to dopa with inhibitor mental side-effects appeared in ten patients in the on-off group including three of those who had developed such symptoms previously, and in 11 in the even group including all five of those who had experienced such symptoms previously i e mental side effects were at least twice as common on treatment with dopa combined with inhibitor as on dopa alone In nine of the patients the combined treatment had to be withdrawn and replaced with dopa alone but in 12 the mental symptoms disappeared on a lower dose of dopa ith inhibitor

Stereotaxic operation Stereotaxic operation had been performed before the start of L-dopa treatment in 15 (35%) of the patients who later developed on-off symptoms but in only four (10%) of those who did not The number of patients who had been operated upon previously was significantly higher ($p<0.01$) in the on-off than in the even group Furthermore a thalamotomy was performed during L-dopa therapy after six and four years of treatment respectively as the second stereotaxic operation in one man with on-off symptoms and as the first operation in one woman in the even group with satisfactory effect on a persisting tremor in both patients The ADL disability as a whole improved only slightly however in the patient in the on-off group and by 1 ADL score in the patient in the "even group No statistically significant difference was found between operated and non operated patients in either of the groups at any time during the L-dopa treatment as regards the ADL disability and the maintenance dose of L-dopa

Anticholinergic drugs Thirty nine of the patients in the on-off group and 41 in the even group had been treated with anticholinergic drugs At the time of the present follow up 35 patients in the on-off group and 29 in the even group were on such treatment No statistically significant difference was found between the patients with and without anticholinergic treatment in either of the groups when correlated to the degree of ADL disability the occurrence of dyskinesia or the presence of dementia

Amantadine chloride Seventeen patients in the on off and 15 in the even group had been treated with amantadine chloride added to the L dopa therapy In some patients amantadine chloride had been added to a L-dopa dose which was lower than that used before, leading to less disturbing involuntary movements but with an antiparkinsonian effect comparably similar to that on the former somewhat higher L dopa dose At the follow up only four patients in each group were still being treated with amantadine the therapy thus having been withdrawn in 24 of the 32 patients The treatment had been discontinued because of lack of effect on the parkinsonian symptoms in seven patients because of transient effect on these symptoms in five and because of mental side effects often including visual hallucinations in 12

Neuroleptic drugs Because it is well known that neuroleptic drugs can produce or impair existing parkinsonian symptoms great care has been taken to avoid such therapy in our patients At the time of the follow up two patients in the on-off group and one patient in the ' even group were treated with trifluoperazin (Terfluzin®) used intermittently in low doses in order to temporarily extinguish dyskinesia One patient in the ' even group was treated with clopenthixol (Sordinol®) in low doses in order to control a latent psychosis Two patients in the on-off group and one patient in the "even" group were given small doses of levomepromazine (Nozinan®) at night in order to induce a quiet sleep

Analyses in cerebrospinal fluid (CSF) During the first year of L-dopa therapy analyses of homovanillic acid (HVA) and 5 hydroxyindoleacetic acid (5-HIAA) in CSF were performed in the parkinsonian patients before the start of L-dopa treatment (17) Analyses of HVA had been performed in 19 patients from the "on-off group and in 22 from the "even" group and showed 10±2 1 and 16±2 6 ng/ml respectively This difference between the groups was not statistically significant ($0.10<p<0.05$) but the

values in both groups were significantly lower ($p<0\ 001$ for each group) than in a normal group of 84 healthy volunteers (44±2 6 ng/ml) (16) 5 HIAA in liquor had been analyzed in 21 patients from the on off group and in 24 from the even group No difference was found between the groups (23±2 0 versus 22±2 5 ng/ml), but the values were significantly lower ($p<0\ 001$ for each group) than in a normal group of 100 healthy volunteers (32±1 0 ng/ml) (16)

DISCUSSION

An initial improvement of varying degree is seen in 70–90% of patients with Parkinson s syndrome treated with L dopa (10 14 18, 28 35) In many patients the improvement appears to be essentially independent of the original severity of the physical disability (14, 18) The present results indicate that L-dopa treatment does not stop the progress of the underlying disease since after long term treatment the disability due to the parkinsonian symptoms was markedly greater (Fig 8) than during the first period of treatment It thus appears that the patients had been given a respite lasting for many years but not lifelong

In the present study it was found that an on-off phenomenon had developed in 50% of the patients treated with L-dopa for five years or more In most studies on off symptoms are reported in 10–20% of the patients (5 8 25 32) Many of the on off patients in the present material had had an initial period with a fairly even response and many also had a favourable effect (Fig 8) As the duration of such an even period varied individually lasting many years in some patients but only a short time in others (Fig 3) the occurrence of an on off phenomenon could not be explained merely by the duration of the L dopa treatment

The risk of developing on off symptoms was found to be higher the younger the patient had been at the début of Parkinson s disease (Fig 5) and at the start of L dopa treatment (Fig 6) It was found that the patients with an even effect during the whole course of treatment besides being older (Fig 4) also had dementia more often than those who developed on-off symptoms These findings may indicate a somewhat different background to the parkinsonian symptoms in the two patient groups with the extent of the neuron degeneration possibly influencing the manifestation of the clinical response A lower mean age in patients with on off symptoms than in other parkinsonian patients treated with L dopa has also been reported by Markham (25) and Sweet and McDowell (32)

During the previous follow up (18) we observed that the occurrence of dyskinesia was positively correlated to a favourable general improvement It is interesting to note that the patients who later developed on off symptoms were those in whom the initial improvement was most marked (Fig 8) and in whom dyskinesia appeared early (Fig 9) Thus, there seemed to be a relationship between a good initial improvement and the appearance of dyskinesia on the one hand and these findings and a later development of an on off phenomenon on the other An early occurrence of dyskinesia might act as a warning of a subsequent on off phenomenon perhaps especially if there has been a marked initial improvement

Furthermore in the previous report dyskinesia was found to be positively correlated to an early occurrence of muscular hypotonia It was found that the presence of both these symptoms in one and the same individual also seemed to imply some risk of an on off phenomenon later

It has been suggested that the occurrence of on off symptoms might be related to overdosage of L dopa (7 8 25 26 29) The results of the present study indicate that a relationship does exist between the magnitude of the dose and the development of an on off phenomenon since the patients in the on off group were found to have had a significantly higher L-dopa dose than those in the even group throughout their treatment (Fig 7) There was no significant correlation between the time when the on-off symptoms began to appear and the magnitude of the dopa dose although the patients with the earliest occurrence of on off symptoms had the highest and those with the latest occurrence the lowest maintenance dose To what extent the adjustment of doses performed when on off symptoms had become manifest were responsible for the lack of such a correlation cannot be determined in the present study However it is tempting to assume that an on off phenomenon developed later in individuals with a relatively low L-dopa dose It may be consistent with such an assumption that the occurrence of an on off phenomenon might be delayed by keeping the dopa dose low enough the guiding principle perhaps being a dose level which avoids dyskinesia At the

same time one naturally has to consider the relationship between the magnitude of the dose and the desired therapeutic effect In some patients a good therapeutic response cannot be obtained without simultaneous occurrence of dyskinesia

It thus seems that the magnitude of the dopa dose has some bearing on the occurrence of an on-off phenomenon However the differences in age and in frequency of dementia between the on off and the even groups suggest that other factors may be involved too The present observations may indicate that a higher dopa dose could evoke on-off symptoms more easily than a lower one in thereto predisposed individuals

The addition of a peripheral dopa decarboxylase inhibitor to dopa represents an important advance in the treatment of Parkinson s syndrome (6 23 27 30 34) as many patients are able to obtain more effective dopa doses than before often due to less disturbing peripheral side effects However there does seem to be an increased risk of central side effects (23 27) which is illustrated in the present report by the higher frequency of mental side effects and the more disturbing dyskinesia when the therapy was changed Furthermore apparently paradoxical effects with aggravated on off symptoms and impaired parkinsonian symptoms were een although the present study does not allow conclusions about the proposition that on-off ptoms could appear earlier during treatment ith dopa combined with inhibitor than with dopa alone (6) When L-dopa is given with inhibitor higher amounts of dopamine are found in the basal ganglia than when it is given alone (11 22) which is generally considered to be associated with the better antiparkinsonian effect and the more disturbing central side effects when dopa is combined with inhibitor However to what extent the occurrence of on off symptoms can also be related to such a finding is not clear When paradoxical effects occur during dopa combined with inhibitor there is also the possibility of other pathogenetic factors to consider For example it is conceivable that under special circumstances such as damage to the blood–brain barrier the inhibitor could pass into the brain during long standing treatment This would cause an inhibition of the cerebral decarboxylation of dopa leading to increasing parkinsonian symptoms besides leading to intervention in the metabolism of other compounds than L-dopa as the inhibitor is not specific (31)

As a stereotaxic operation had been performed previously in a significantly higher number of patients in the on off than the even group one might suggest that a thalamotony can predispose to the development of on-off symptoms during L dopa treatment However, it is also possible that the operation itself did not alter the response to L-dopa treatment to any appreciable degree but that the indication for the operation had been the presence of a genuine Parkinson s syndrome with its specific neuron damage which might be a prerequisite for on off symptoms

It is tempting to relate the present results to certain pharmacological findings Normally dopamine like other monoamines is concentrated in special granules (19, 20) in the nerve terminals which location seems to be a prerequisite for release by nerve impulses (2 20 24) Even at an early stage of Parkinson s syndrome the dopamine formed from L dopa might be released in this physiological manner (1 4) In later stages when the disease has progressed the number of nerve cells is further reduced but as there is a relative excess of dopa decarboxylase dopamine will easily be formed when L-dopa is given This will imply quantities of dopamine that are too large to be taken up by the remaining granules so that large parts are apparently found instead in the cytoplasma in the nerve terminals leaking to the postsynaptic receptors without obvious influence of nerve impulses (4) Such an uncontrolled release of dopamine might lead to a sudden onset and cessation of the effects of L dopa possibly explaining the occurrence of on-off symptoms (4) Furthermore when presynaptic neurons degenerate a postsynaptic denervation supersensitivity (3 33) could be present which might not only facilitate the antiparkinsonian effect of L-dopa but also contribute to the appearance of dyskinesia (4) With high doses of L-dopa leading to larger amounts of dopamine these effects could be expected to occur to a greater extent (1 4) These findings are in agreement with the high dopa doses, the often marked initial improvement during L dopa therapy and the early occurrence and high percentage of dyskinesia found in the patients who later develop on-off symptoms

Thus it could be assumed that a good and fairly even initial effect during L-dopa treatment in presumptive on-off patients might be connected with the parkinsonian disease being in an early stage the remaining neurons being able to function almost

adequately The development of on off symptoms during L dopa treatment seems to represent a more advanced stage of the disease with a progression of the specific and localized damage of the neurons in the basal ganglia and where the magnitude of the L dopa dose seems to have some bearing on the appearance of such symptoms As the patients who maintained an even symptomatology during long term treatment with L dopa were older and in particular as they had a higher frequency of dementia than those developing on off symptoms the possibility of a more widespread neuron damage influencing the clinical manifestation in those patients has to be taken into consideration

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Four Cases of Long-Standing Diarrhoea and Colic Pains Cured by Fructose-Free diet—A Pathogenetic Discussion

Dan E H Andersson and Arne Nygren

From Medical Department II Sodersjukhuset Stockholm Sweden

ABSTRACT Four patients with a long history of abdominal swelling, colic pains and diarrhoea were cured by a fructose free diet They were all given an oral load of (^{14}C) fructose and their expiratory excretion of $^{14}CO_2$ was found to be significantly lower than that of control patients with similar symptoms It is concluded that the patients cured by a fructose free diet might have a partial fructose malabsorption

Patients with long standing intermittent abdominal complaints like swelling flatulence colic pains and diarrhoea are often seen in medical practice Sometimes these symptoms can be explained on the basis of impaired hydrolysis and absorption of carbohydrates due to intestinal enzyme deficiencies or lack of a specific transport system Lactase deficiency is the most common example of such a disturbance (11)

In this study we present four patients with a case history which suggested that foods containing fructose may have provoked their abdominal symptoms When these patients were given a fructose free diet they experienced a clear cut improvement In these patients we have studied several biochemical variables after an oral fructose load and compared the results with those for ten control patients with similar symptoms Three of the control patients experienced symptoms when challenged with fructose but were not cured by a fructose free diet

METHODS

All tests were performed in the morning after an overnight fast (10–12 hours) Blood samples were drawn from an antecubital vein through an indwelling catheter which was kept open by a slow i v drip (isotonic NaCl)

Fructose (100 g) was dissolved in 400 ml water and ingested by the patient over a period of not more than 5 min Glucose was determined according to a glucose oxidase method (Glox Kabi Stockholm Sweden) Fructose was assayed by an enzymatic method using hexokinase and glucose 6-phosphate-dehydrogenase (4) Lactate was determined with a commercial kit (5)

A biopsy specimen from the small intestine (close to the ligament of Treitz) was taken perorally with a Carrey capsule (8) and immediately examined in a dissection microscope Thereafter the specimen was homogenized in a hand glass homogenizer (Potter and Ölvehjelm) containing 1 ml 0 15 M ice-cold KCl The homogenate was not centrifuged prior to analyses All analyses were performed on the same day The protein content in the homogenate was analysed according to Lowry et al (15) Fructose 1 6-phosphate aldolase activity was determined with a commercial test kit (6) Fructose 1 phosphate aldolase activity was assayed according to Wolf and Lenthardht (3) Fructose 1 6-phosphate activity was measured as described by Baker and Winegrad using a 50 mM glycerin buffer with pH 9 4 (2)

$^{14}CO_2$ breath analysis after peroral (^{14}C) fructose administration

Five μCi (^{14}C) fructose (U) together with 50 g fructose was dissolved in 500 ml water and given as an oral load The specific activity of CO_2 in breath was measured (before and at 15 30 45 60 90 120 150 and 180 min after (^{14}C) fructose intake) according to the method described by Abt and von Schuching (1) The exhaled air was first blown through self indicating silica gel The CO_2 in the exhaled air was then trapped in a scintillation vial containing 1 ml 1 M hyaminehydroxide in methanol (1 1) and 1 ml 96% ethanol together with phenolphtalein (1%) as indicator The blowing was continued until the solution became colourless indicating that 1 mmol of CO_2 had been trapped After adding 15 ml scintillation cocktail (Instagel/1 M HCl 9 1) the sample was counted in a liquid scintillation counter (Paccard Tri Carb) Counts were converted to disintegration per min (d p m) by external standard

In order to determine the endogenous output of CO_2 the air excreted during 6 min was collected in a Douglas bag The air volume was measured and the CO_2 concentration was determined according to the method described by Scholander (20) The endogenous output was determined immediately before as well as 60 120 and 180 min after the (^{14}C) fructose intake The ouput of CO_2 at

Table I *Vital data on the patients investigated*

Pat no	Sex	Age (y)	Weight (kg)	Height (m)	Diagnosis
Group I					
1	♂	68	90	1 77	
2	♀	22	55	1 67	
3	♀	20	68	1 69	
4	♂	56	74	1 67	
Group II					
5	♂	29	67	1 73	Lactase deficiency
6	♂	38	65	1 84	Lactase deficiency
7	♀	41	66	1 63	Lactase deficiency
8	♀	50	76	1 63	Lactase deficiency
9	♂	22	70	1 74	Ulcerative proctitis
10	♂	45	74	1 84	Irritable colon
11	♀	50	55	1 60	Irritable colon
12	♀	41	58	1 75	Irritable colon
13	♂	32	75	1 83	Irritable colon
14	♀	38	50	1 52	Irritable colon

other points of time was estimated from a curve obtained with the above mentioned values

Calculations The excretion of $^{14}CO_2$ was expressed as a percentage of the administered (^{14}C) dose exhaled per min This was obtained as follows The specific activity of expired $^{14}CO_2$ in each sample (d p m /mmol CO_2) was divided by the dose administered (d p m) and then multiplied by the endogenous output of CO_2 in the excreted air (mmol/min) The cumulative $^{14}CO_2$ excretion was obtained by calculating the individual areas under the $^{14}CO_2$ excretion curves Student s *t* test was used for stical evaluation

SUBJECTS

All patients displayed intermittent intestinal symptoms (colic pains flatulence and diarrhoea) Vital data are given in Table I The patients were divided into two groups those who became free from symptoms when given a diet free from fructose (group I) and those who served as controls (group II) Before the study all patients gave their informed consent to participation

Group I Patients 1–4 had for a long time experienced periods of abdominal bloating and distention colic pains and diarrhoea Between these periods which lasted for 2–6 weeks they had only slight gastrointestinal complaints All had noticed an aggravation of symptoms whenever they had eaten fruits or food containing appreciable amounts of cane sugar All patients were free from symptoms on a fructose free diet Patient 1 has been on such a diet for five years patient 4 for three years patients 2 and 3 for two years

During the year before the fructose free diet was started the following tests had been performed with normal results In patients 1 and 2 Schilling test vitamin A absorption test lactose tolerance test radiological examination of the stomach jejunum ileum and colon A jejunal biopsy specimen showed normal villi In patient 3 lactose tolerance test jejunal biopsy and radiological examination of the gallbladder and colon In patient 4 vitamin A absorption test lactose tolerance test jejunal biopsy and radiological examination of the stomach and colon Before the fructose free diet was introduced radiological examination of the jejunum and ileum showed that a short segment of the latter was dilated and remained distended for 30 min i e findings consistent with a stagnant loop syndrome The level of vitamin B_{12} in serum was 47 pmol/l (normal level 100–700) A Schilling test without intrinsic factor showed a value of 8 6% No vitamin B_{12} therapy or other medical treatment was given with the fructose free diet Three years later radiological examination of the jejunum and ileum was normal Vitamin B_{12} in serum was 256 pmol/l and a Schilling test without intrinsic factor showed a value of 15 6% Patients 1–4 had been given a fructose free diet and were without symptoms when the $^{14}CO_2$ breath test was performed

Group II The control patients (nos 5–14) had been troubled for years by periods of diarrhoea and abdominal pains Patients 5–8 proved to be lactose intolerant and were essentially without symptoms on a lactose free diet Patient 9 had ulcerative proctitis In patient 10 a xylose tolerance test a lactose tolerance test a vitamin A absorption test faecal fat content and radiological examinations of the stomach jejunum and ileum were all normal Patient 11 had undergone salpingo-oophorectomy and hysterosalpingectomy because of ovarian cysts and uterine myoma After that she was troubled by colic pains

Table II *Fructose (mean and range) glucose and lactate levels (mean ± S D) in blood in groups I and II after an oral fructose load of 100 g*

Time (min)	Fructose (mmol/l)		Glucose (mmol/l)		Lactate (mmol/l)	
	Group I	Group II	Group I	Group II	Group I	Group II
0	0	0	4 2±0 2	4 3±0 6	1 0±0 1	1 1±0 3
15	–	–	4 7±0 2	4 6±0 4	1 3±0 3	1 5±0 5
30	0 4 (0 2–0 5)	0 5 (0 2–0 9)	4 8±0 2	4 9±0 6	1 9±0 4	2 2±0 5
60	0 4 (0 3–0 6)	0 6 (0 3–0 8)	4 2±0 5	4 8±1 1	2 6±0 4	2 6±0 5
90	–	–	4 1±0 4	4 3±0 6	2 5±1 0	2 4±0 5
120	0 4 (0 2–0 4)	0 5 (0 2–0 7)	4 4±0 3	4 4±0 4	2 3±0 6	2 1±0 4
150	–	–	4 3±0 2	4 3±0 4	2 2±0 5	1 8±0 4
180	–	–	4 4±0 2	4 4±0 5	1 8±0 4	1 4±0 4

Table III *Endogenous CO_2 output in breath in groups I and II after an oral fructose load of 50 g (mean and range)*

Time (min)	Breath CO_2 (mmol/min/kg)	
	Group I	Group II
0	0 148 (0 098–0 187)	0 154 (0 085–0 258)
60	0 175 (0 153–0 193)	0 179 (0 121–0 244)
120	0 151 (0 124–0 187)	0 158 (0 095–0 248)
150	0 129 (0 103–0 148)	0 149 (0 092–0 240)

and diarrhoea A third laparotomy was performed and showed intestinal adhesions Radiological examinations of the gallbladder stomach jejuno-ileum and colon were normal A lactose tolerance test was normal Patients 12 and 13 had suffered for years from colic pains and diarrhoea alternately with obstipation Radiological examinations of the jejunum ileum and colon and a lactose tolerance test were normal Jejunal biopsy showed normal villi Patient 14 had had watery diarrhoea and colic pains for 2 years Lactose tolerance test vitamin A absorption test and radiological examination of the jejunum ileum and colon were normal

Patients 12–14 had some abdominal discomfort after the fructose tolerance test When given a fructose free diet for three months no change was detected in their abdominal symptoms At the time of the $^{14}CO_2$ breath analysis patients 5 7 9 and 13 were essentially without symptoms while the other patients in this group all had mild to moderate diarrhoea

RESULTS

Peroral fructose tolerance test (100 g)

In patients 1–4 (group I) the test produced intensive colic pains abdominal swelling and diarrhoea Some control patients (nos 12–14) also had abdominal discomfort after the test There were no significant differences in the fructose glucose or lactate levels between the two groups (Table II)

Endogenous CO_2 output

There were no significant differences between the two groups but large variations occurred within each group (Table III)

$^{14}CO_2$ breath test

At 30 45 and 60 min after the peroral (^{14}C) fructose intake the amount of $^{14}CO_2$ exhaled was significantly lower in the patients in group I than in group

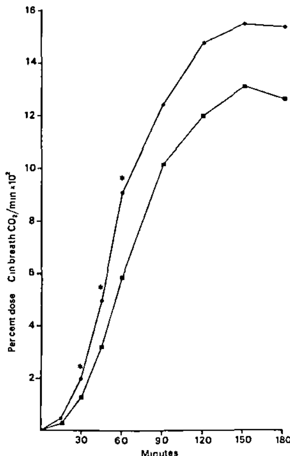


Fig 1 Mean values for percentage of administered (^{14}C) fructose excreted as $^{14}CO_2$ in breath after ingestion of 50 g (^{14}C) fructose ■—■=Group I ●—●=group II *$p<0.001$

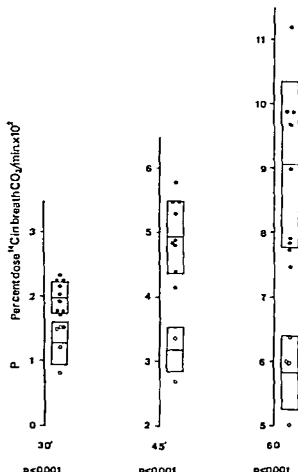
II ($p<0.001$) (Fig 1) At 45 and 60 min there was no overlapping (Fig 2) When the individual areas under the $^{14}CO_2$ excretion curves were calculated (giving the cumulative excretion of $^{14}CO_2$) significant differences between the two groups were found for the periods 0–30 to 0–150 min after (^{14}C) fructose ingestion (Fig 3)

Enzyme activities in homogenates of jejunal mucosa

Activities of the assayed enzymes were found in all cases There was a considerable overlap in the activities between the groups (Table IV)

DISCUSSION

In this series of cases with intermittent intestinal symptoms such as colic pains flatulence and diarrhoea four patients (nos 1–4) became



2 Individual values for percentage of administered (^{14}C) fructose excreted as $^{14}CO_2$ in breath after ingestion of (^{14}C) fructose Bars represent mean ±S D O=Group ●=group II

symptom free when given a diet devoid of fructose These four subjects had a lower expiratory excretion of $^{14}CO_2$ than the other patients

Several factors are known to influence the $^{14}CO_2$ excretion in breath after (^{14}C) fructose intake Thus the total output of CO_2 in breath varies with the patient s nutritional state and metabolic activity (21) Most authors dealing with $^{14}CO_2$ breath tests have assumed the endogenous CO_2 output to be constant (0 141 mol/min/kg) supposing that the error involved in using such a constant is without importance in most patients (14) This was not the case in our patients who displayed a wide range of endogenous CO_2 output. But as the actual endogenous CO_2 output was used in the present calculations the intergroup differences in $^{14}CO_2$ excretion cannot be explained by differences in the total CO_2 output in breath

The $^{14}CO_2$ in breath after administration of ^{14}C labeled materials is affected by the kinetics of the CO_2–HCO_3 pools which the labeled CO_2 must traverse before its excretion in breath (23) We can not exclude the possibility that the CO_2–HCO_3 kinetics varied among our patients In the absence of obesity and endocrine disorders however it is unlikely that such differences can account for the lower $^{14}CO_2$ output in breath in our group I A delayed gastric emptying might have caused the low $^{14}CO_2$ excretion during the first part of the test in group I However the intestinal symptoms after fructose intake and the benefit of a fructose free diet in these patients are difficult to explain on the basis of a delayed gastric emptying

After absorption fructose is largely metabolized by the liver (9) Thus a less effective hepatic uptake and combustion of fructose might explain the lower $^{14}CO_2$ excretion in group I If so one would expect a higher blood fructose level in group I than in group II In fact the fructose level tended to be lower in group I

Another explanation for the lower $^{14}CO_2$ excretion might be an incomplete absorption of fructose in the small intestine in patients 1–4 However in the latter part of the test there was no significant difference in $^{14}CO_2$ excretion In this context it is of interest that in patients with lactase deficiency the $^{14}CO_2$ excretion is lower than in healthy subjects only during the first hours after a (^{14}C) lactose intake (16) There is evidence indicating that unabsorbed disaccharides in the colon are degraded by bacterial fermentation and the $^{14}CO_2$ so formed is

Table IV *Enzyme activities in jejunal mucosal homogenates expressed as μmoles of substrate metabolized per min per mg protein*

Pat no	Fructose 1-6-diphosphate	Fructose 1 phosphate aldolase	Fructose-1-6-diphosphate aldolase
Group I			
1	13	6	16
2	30	21	41
3	40	13	–
4	56	20	51
Group II			
5	38	16	18
6	11	10	27
12	17	14	35
13	18	21	43

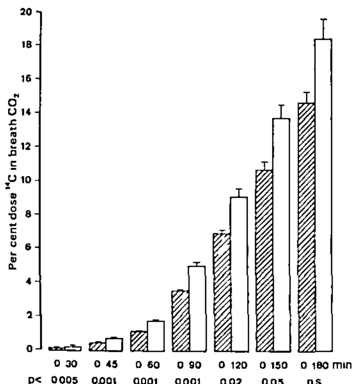


Fig 3 Cumulative $^{14}CO_2$ excretion (mean ±S E M) in breath as a percentage of dose administered after ingestion of 50 g (^{14}C) fructose ▨=Group I □=group II

excreted in breath (7) so it seems possible that the low $^{14}CO_2$ excretion during the initial part of the breath test reflects an impaired absorption of fructose in the small intestine

For a long time it was thought that fructose is absorbed only by diffusion through the small intestinal wall (10) There is now evidence that a system exists for an active fructose absorption (13) Perhaps our patients have a disturbance in this postulated system It seems unlikely that the intestinal symptoms in our patients would have been elicited by a minor reduction in fructose absorption per se

Other factors which might have contributed include a toxic effect of fructose upon the small intestine due to a defect in the intestinal fructose metabolism In subjects with hereditary fructose intolerance an oral fructose intake results in diarrhoea abdominal pains and hypoglycaemia Moreover high fructose and lactate levels are found in blood after fructose ingestion (12) The biochemical findings in our patients after fructose administration rule out the diagnosis of hereditary fructose intolerance Activities of fructose diphosphate aldolase fructose 1 phosphate aldolase and fructose diphosphatase were found in all homogenates of the jejunal mucosa It is known that the activities of glycolytic enzymes in the intestinal mucosa are highly dependent upon several factors such as previous carbohydrate intake folic acid administration site and depth of the biopsy (17 18) Therefore it is not possible to draw any firm conclusion as to whether or not the present activities are within the normal range However our findings do exclude a deficiency of the assayed enzymes as a cause of the symptoms in our patients

Schneider and Gunther (19) described a patient with lactase deficiency and gastrointestinal symptoms after fructose intake This patient had a high blood fructose after fructose intake in contrast to our patients Moreover all patients in our group I had a normal lactose tolerance test In case 4 a Schilling test performed prior to the fructose free diet showed vitamin B_{12} malabsorption In cases with bacterial overgrowth in the small intestine treatment either surgically or medically (e g oral broad spectrum antibiotics) has been found to improve the absorption of vitamin B_{12} (22) A normal Schilling test was obtained in case 4 after the fructose free diet had been given as the only treatment

An altered bacterial growth in our patients could

have been promoted by a constant leakage of un absorbed fructose to the distal part of the ileum An altered bacterial flora in the colon and perhaps also a bacterial colonization of the small intestine may have contributed to elicit the symptoms

We tentatively postulate that the low $^{14}CO_2$ excretion found in group I reflects a reduced absorption of fructose which together with other factors possibly bacterial colonization in the small intestine may have produced the symptoms Perhaps such a mechanism is involved in the production of symptoms in many patients with intermittent diarrhoea and colic pains

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Increase in Serum Alkaline Phosphatase (S-ALP) in Chronic Myelocytic Leukemia—Sign of Drug-Induced Cholestasis?

Robert Hast

From the Department of Medicine Division of Haematology Karolinska Hospital Stockholm Sweden

ABSTRACT In four patients with chronic myelocytic leukemia, a solitary rise in serum alkaline phosphatase (S ALP) was noted 2–12 months prior to death All patients had received busulphan (Myleran®) therapy for longer than 12 months (total dosage 1 0–2 4 g) It is suggested that the increase in S ALP was due to a cholestatic liver damage, possibly secondary to the busulphan treatment

All cytotoxic agents must be expected to cause liver injury if sufficiently large amounts are given (3) Nevertheless hepatotoxicity is not a frequently reported side effect except for the antimetabolites (4) Among alkylating agents there are only occasional reports on liver toxicity (2)

PATIENTS

In four patients with chronic myelocytic leukemia (CML) a significant rise in serum alkaline phosphatase (S-ALP) was noticed during the late phase of the disease In retrospect it was found that the increase in S ALP in all four cases could be traced back 2–12 months It was not possible to say whether the rise in S-ALP had a gradual or a sudden onset since the enzyme had not been checked regularly in the past (Fig 1)

Case 1

A 40-year-old man with CML since April 1972 who was treated with busulphan in periods (total dose 1 0 g) until the terminal phase in Jan 1974 Splenectomy was done in Oct 1973 mainly because of thrombocytopenia and major hemolysis S ALP was within normal limits initially but rose during the last 4 months from 6 to 12 times the upper normal value Transaminases were only slightly increased Autopsy showed an enlarged liver with diffuse leukemic infiltration signs of intrahepatic cholestasis and cholelithiasis There was no obstruction of the common biliary duct

Case 2

A 53 year old woman with CML diagnosed in 1968 Until 1972 only radiation therapy to the spleen had been given Then busulphan was added in intermittent dosage and given until the end of 1973 (total dose 2 4 g) During the terminal phase which was not a blastic crisis hemorrhage and septicemia supervened Splenectomy was done because of pains and discomfort S-ALP rose up to 10 times the normal limit during the last months The transaminases were virtually normal Electrophoretic separation showed high α 1 and α 2 fractions of S-ALP Histopathological examination at autopsy showed only minor leukemic infiltration of the liver

Case 3

A 41 year-old housewife with CML since 1970 who had been treated with busulphan in periods (total dose 2 0 g) The spleen was moderately enlarged but the liver was

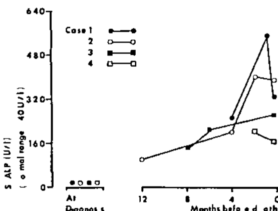


Fig 1 Increase in S-ALP in four cases of CML treated with busulphan

normal S ALP which was normal in 1970 began to rise in Jan 1973 up to a sixfold increase before and during the terminal blastic phase in Sept 1973 The transaminases were only slightly increased Electrophoresis of S ALP showed increases in the α 1 and α 2 fractions Autopsy revealed signs of intense leukemic infiltration in the liver

Case 4

A 25 year-old housewife in whom the diagnosis of CML was established in 1972 Busulphan was given in intermittent dosage for 20 months (total dose 1 4 g) and the patient remained well until the terminal blastic phase S ALP showed a fivefold increase during the last 2 months while transaminases were normal Examination of the liver at autopsy showed neither cholestasis nor blast cell infiltration but the postmortem changes were quite extensive

DISCUSSION

Busulphan (Myleran®) is a widely used drug for the control of CML The spectrum of side effects has recently been reviewed (1) Some, like aplastic anemia and pancytopenia are directly related to drug toxicity Others like pulmonary fibrosis and Addisonian pigmentation are more puzzling They appear in a few maybe sensitized patients and are not related to the total dosage of busulphan

So far there has been only one report on he patotoxicity associated with busulphan treatment (5) Underwood et al described a 25 year old man ith CML treated with busulphan for more than x years (total dose 4 075 g) who developed holestatic jaundice during the terminal phase of the disease

In the present study all patients had received busulphan intermittently or continuously for more than 12 months The total dose given ranged from 1 0 to 2 4 g The up to 12 fold increase in S ALP was not accompanied by any extensive transaminase increase Indeed S ALP can be of intestinal hepatic as well as osteogenic origin but electrophoretic separation of S ALP showed creased α 1 and α 2 fractions, a pattern typical enzymes released by the liver and biliary tract

Among patients with malignant tumors, a sol increase in S ALP might be an early sign of ca metastasis to the liver (3) In theory this might apply to cases of leukemia, but in our experie patients with acute leukemia do not show increa S ALP, despite blast cell infiltration of the li Furthermore when the signs of cholestasis w first noted 2–12 months prior to the terminal ph (Fig 1), all patients were in a good clinical co tion with no signs of blastic transformation

The histological examination of the liver at topsy showed intrahepatic cholestasis in one (only and varying degrees of leukemic infiltra and postmortem changes in the others Defi conclusions regarding the etiology of cholest could not be made, but it is suggested that increase in S ALP in these four patients was cau by the busulphan therapy Further studies on liver function in patients on long term treatm with busulphan will be of interest

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Population Studies on Non-Obstructive Urinary Tract Infection in Non-Pregnant Women Importance of Method and Material

Evaluation of 844 Women and 232 Male Controls

Nils Alwall

From the Health Service of Malmohus County District University Hospital Lund Sweden

ABSTRACT In earlier population studies other workers found no significant differences in the blood urea levels between bacteriuric non pregnant women and their controls matched for age or for age, civil status, and maternity From these results, far reaching negative conclusions were drawn about the importance of urinary tract infection (UTI) and the value of early diagnosis and screening for bacteriuria It was also concluded that 'asymptomatic bacteriuria" is not truly asymptomatic From a medical examination, patients with obstructive uropathy, concretions, diabetes mellitus, possible glomerulonephritis, and other parenchymal diseases were excluded from the present study The investigation comprised 232 male controls and 844 non pregnant women, aged 21–70 years, who were subjectively asymptomatic at the time of examination The women were divided into four study series, which were uniform with respect to age and maternity 1) Controls with no past history of upper or lower UTI and no urinary abnormalities i e neither bacteriuria nor pyuria 2) Women with a past history of symptomatic UTI but no urinary abnormalities at the time of examination 3) Women with sterile pyuria 4) Women with bacteriuria/pyuria About 40% of the women in the latter two series had no past history of UTI Thus, asymptomatic" bacteriuria does exist The concentrations of serum urea N (SUN) and serum creatinine did not differ significantly between the four series of women In each series both levels increased similarly with increasing age (ageing") There was no significant difference in the women with bacteriuria before and after elimination of the infection by treatment The levels did not rise with increasing age in the male control series and were significantly higher than in the corresponding female control series In contrast, the level of maximal urinary concentrating ability was significantly *higher in the female series 1 than in series 2* There were no significant differences between women in series 2 and either series 3 or 4 Furthermore, in series 3 and 4 there were no significant differences between women with and without a past history of UTI Elimination of the bacteriuria was accompanied by improved concentrating ability in series 4 The groups with sterile pyuria and bacteriuria/pyuria, respectively who had no past history of UTI had significantly lower osmolality than the controls There was a significant difference in the age related reduction of concentrating ability between the control series and the other female series ("ageing ') which showed the greatest decrease The level was higher in the male than in the female control series, the differences in the regression coefficients being significant and in the intercepts not significant Consequently, compared with the SUN and serum creatinine concentrations, the maximal concentrating ability seems to be the method of choice in population surveys of this kind It is essential however, that the test should be carried out under current standardized conditions Initial diuresis provoked by oral intake of 20–40 mg of furosemide (Lasix®) followed by fluid deprivation will greatly increase the percentage of technically satisfactory tests Radiological abnormalities suggestive of 'chronic pyelonephritis" were noted at i v urography in the same prevalence, 11 0, 8 1 and 9 3%, respectively, in the female series 2, 3, and 4 Thus, contrary to conclusions by others, it cannot possibly be inferred that bacteriuria per se is necessarily related to the appearance of acquired abnormalities of this type unless it is assumed that the women in series 2 and 3 had antecedent bacteriuria There was an age-related continuous increase in such acquired abnormalities in the various female age groups, from 1 2 to 11 8% Further studies of this kind will be necessary to define any special groups at risk

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Paper read in part at the International Workshop on Urinary Tract Infection in Rostock May 4 1975 organized by the Society of Nephrology of the GDR and sponsored by the International Society of Nephrology

The traditional clinical opinion that bacteriuria is a symptom of disease requiring treatment has been manifested by innumerable publications In recent years the question has become controversial especially as regards bacteriuria in non pregnant women In a recent Editorial with a summary of his own experience and that of others Asscher (7) concluded that There is no evidence that untreated significant bacteriuria in the adult produces progressive kidney damage provided the bacteriuria is not associated with raised blood pressure or obstructive uropathy that early diagnosis of urinary tract infection (UTI) is of limited value and that screening of non pregnant women for bacteriuria is not justified Freedman (12), in a recent survey discussed the clinical diagnosis of interstitial nephritis and concluded that it is easy to identify the primary association of many factors with this renal disease but urinary tract infection does not seem to be one of them

Both authors based their conclusions on the argument that in population studies they had not found any significant difference in the levels of blood urea between bacteriuric non pregnant women and their matched controls

The urinary concentrating ability test was used in two population surveys which will be referred to in the following It has long been known that the concentrating ability is one of the kidney functions that affected early during acute pyelonephritis in children and adults (9 16) and in asymptomatic bacteriuria in pregnant women (14) In chronic pyelonephritis there is a disproportionate decrease in concentrating ability compared with glomerular filtration rate (9)

Asscher and his group (7 8) found no difference in the maximal concentrating ability in non pregnant women who had a history of frequency and dysuria at any time and women with no such history Asscher postulated that there is approximately a 50% chance that women with such symptoms will also have bacteriuria and concluded that there is no evidence that bacteriuria does damage I found however a reduced concentrating ability in non pregnant women with a history of UTI as compared with women without such a history (2) and emphasized that the difference in the results between the two population surveys might be ascribed to insufficient water deprivation in the former

The object of the population study presented here was to provide a basis for re evaluating some of the facts and conclusions quoted in the foregoing and the interaction of basic factors that might not have been sufficiently considered The present material was divided into series which allowed analysis of the importance of a past history of UTI and normal and abnormal urinary findings not only of bacteriuria but also of pyuria A comparison could thus also be made between the results of several tests of kidney function and it was possible to study the relationship between radiological abnormalities of pyelonephritis type and factors other than bacteriuria Finally, the kidney function in male and female controls with no urinary abnormalities and no past history of UTI was compared

STUDY POPULATION AND METHODS

Previous papers (2 4) have given some results obtained in a computer selected population of 3 998 persons 21-69 years old who were screened for UTI in 1969-71 Since only 56 4% of those invited attended the screened persons do not represent a cross section of the total population Between 1973 and 1975 the material was augmented by adding women with sterile pyuria and bacteriuria/pyuria and women with no urinary abnormalities who had a past history of UTI These consecutive cases were partly included in a series of women who were screened for gynaecological cancer and UTI

A questionnaire on past history of UTI etc was sent to 1 959 men and 2 039 women aged 21-69 in connection with screening for UTI in the period 1969-71 The questions referred to disorders of upper UTI type (loin pain and fever) lower UTI (dysuria and frequency lasting for 24 hours or more) a few or several periods of UTI heredity for hypertension and/or current treatment for the latter disease At the time of the medical examination the reliability of the written answers was checked by oral questioning in which the subjects did not have access to their own completed questionnaires The deviation was less than 0 3% for points of importance in this connection. Most subjects with upper UTI had co-existing symptoms of cystitis

Medical examination The subjects were selected in 1969-75 in connection with the medical examination.

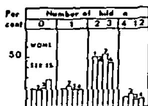


Fig 1 Percentage of women in series 1 2 3 and 4 with no children one child two or three children and four to twelve children

Those who at the time had subjective symptoms of urinary tract disease or were undergoing treatment for UTI were excluded as were those in whom medical examination showed clear evidence of obstructive disease of the urinary tract genital prolaps urolithiasis diabetes mellitus analgesic abuse possible glomerulonephritis or other known forms of parenchymal renal disease

Altogether 844 non pregnant women were divided into the following series 1) 324 controls with no urinary abnormalities who had no past history of UTI 2) 172 women with no urinary abnormalities who had a past history of upper and/or lower UTI 3) 206 women with sterile pyuria including 87 (42 2%) who had not and 119 (57 8%) who had had UTI in the past and 4) 142 women with bacteriuria and pyuria including 57 (40 1%) who had not and 85 (59 9%) who had had UTI in the past Of the women with bacteriuria 97 4% also had pyuria diagnosed according to the criteria defined later in this paper The mean ages of the four series were 43 7 43 6 46 2 and 45 0 years respectively

Fig 1 shows the percentage distribution within the four series of the following groups women with no child with one child 2 or 3 children and 4–12 children The differences were not significant

The male control series comprised 232 persons their mean age was 44 9 years They had no urinary abnormalities and no past history of UTI

As a rule the subjects were called to a medical examination successively from different areas of the county district The study was discontinued in Oct 1975 as further resources were no longer provided

Intravenous urography was carried out in consecutively selected cases with as far as possible an even age distribution Owing to insufficient resources the number of examinations had to be limited in the control series to 32 4% of the women and 34 1% of the men The mean age of the female controls in series 1 was 44 0 years and of the males 52 1 years (in the whole series 43 7 and 44 9 years respectively) In the female series 2 70 3% were examined and their mean age was 45 1 years (43 6 years) In series 3 the corresponding figures were 65 1% and 46 7 years (46 2 years) and in series 4 91 0% and 45 2 years (43 4 years) The latter series of patients with bacteriuria/pyuria includes 99 persons from the series of 102 cases reported earlier (2) all of whom were examined by i v urography Three patients were excluded because of diseases diagnosed later vesico-ureteral reflux (two cases) and diabetes mellitus (one case) Owing to lack of resources all of the women added to the series during 1973–75 could not be examined

In series 3 (pyurics) the figures concerning percentage of those examined and mean age were 49 4% and 47 9 years (45 4 years) for the subgroups with no past history of UTI and 67 2% and 46 1 years (46 7 years) for the subgroups with such a history In series 4 (bacteriurics/pyurics) the corresponding figures were 86 0% and 47 7 years (45 1 years) for the former and 94 1% and 45 2 years (43 4 years) for the latter

Micturition urethrocystography was carried out in 66 0% of the bacteriurics/pyurics but the results will not be reported here since the number of examinations in the rest of the series was too low to allow a comparison

Criteria for the diagnosis of bacteriuria Significant bacteriuria with the same species in at least three clean voided midstream collections was required The dip-slide inoculated at the medical examination in our dispensary gave results that agreed closely with parallel pour plate viable counts When the nitrite test was positive there was good agreement as regards simultaneous findings of bacteriuria in the voided sample and suprapubic urine (5) The combination of the dip-slide technique and the nitrite test is diagnostically valuable

Criteria for the diagnosis of pyuria More than 4 WBC per high power field (dry objective magnification×320) in two or more consecutive clean voided midstream urine collections with our technique roughly equivalent to 40 cells/mm³ (1)

Serum urea N (SUN) and serum creatinine Routine methods (Central Chemical Laboratory of the University Hospital) When the blood was sampled in the morning the subjects had taken no fluid in preparation for the concentration test

Maximal concentrating ability Urine osmolality was determined by freezing point depression using a standard osmometer The invitation to the medical examination was accompanied by written instructions to withhold the intake of fluids or fluid rich food as from noon on the day preceding examination otherwise usual diets Urine was collected overnight (about 7 p m –7 a m) for measurement of urine volume and urine osmolality If the over night volume exceeded 300 ml fluid was withheld for an additional four hours If urine osmolality did not exceed 800 mOsm/kg the test was repeated if necessary after an i m injection of Pitressin tannate 5–10 U in oil (16)

Improved preparations for the concentration test The persons who were invited to take part in the medical examination in the last 2–3 years of this survey also received a letter containing one tablet 40 mg of furosemide (Lasix®) to be taken by mouth at about 8 or 9 a m on the day preceding the medical examination that is 3–4 hours before they started to withhold the intake of fluid and fluid rich food They were instructed to restrict the dose to half a tablet of furosemide or to divide the dose taking first half a tablet (20 mg) and after a few hours the other

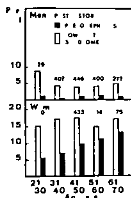


Fig 2 Percentage of men and women in different age groups with a past history of upper and/or lower UTI

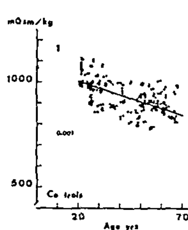


Fig 3 Significant age related decrease in maximal urinary concentrating ability in the female series 1 (controls with no urinary abnormalities i e neither pyuria nor bacteriuria/pyuria and no past history of UTI) ($r = -0.50$ $p<0.001$)

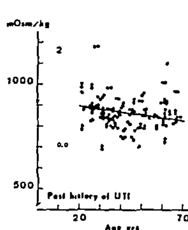


Fig 4 Significant age related decrease in maximal concentrating ability in series 2 (women with no urinary abnormalities and a past history of UTI) ($r = -0.20$ $p<0.01$)

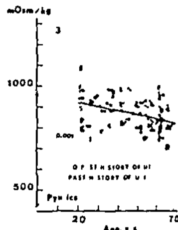


Fig 5 Significant age related decrease in maximal concentrating ability in series 3 (women with sterile pyuria with and without a past history of UTI) ($r = -0._7$ $p<0.001$)

half if the subsequent diuresis could be expected to interfere with their work or other activities In only a few persons did half a tablet cause such a heavy diuresis that they refrained from taking the second half

This provoked initial diuresis together with restricted fluid supply as instructed greatly increased the reliability of the test and can be recommended in carrying out the concentration test in ambulant medical practice The need for repeated tests was thus reduced from 25 to 4% in a ... of two series each comprising 300 consecutive ex...ations Few subjects complained of troublesome ...uresis and/or thirst and/or slight orthostatic disorders The latter complication occurred mainly in subjects who had earlier been troubled with orthostatism and could in most cases be prevented by an instruction to limit the furosemide dose to half a tablet (20 mg)

RESULTS

Presence of a past history of UTI in a screened population

Fig 2 shows data on a past history of UTI in the questionnaire sent to 1959 men and 2039 women aged 21–69 in connection with screening for UTI in 1969–71 In the men the figures for a past history of upper and lower UTI are fairly low and constant in the age groups above 30 about 1% and 3% respectively Elderly men with disorders that could be due to hyperplasia of the prostate were excluded The corresponding figures for women showed an increase in the proportion of upper UTI (and usually co existing lower UTI) from about 5% to about 14% with increasing age, the frequency of data on lower UTI was approximately the same in all age groups (about 15–17%) These figures are of interest as a basis for the discussion of the results of the medical examination

Maximal concentrating ability

Figs 3–6 show the relation between osmolality and age in the individual cases in the four series of non pregnant women namely series 1 and 2 women who had no urinary abnormalities without and with a past history of UTI respectively series 3 women with sterile pyuria and series 4 women with bacteriuria (97 4% having pyuria as well) About 60% of the women in the latter two series had a past history of UTI Maximal concentrating ability decreased with increasing age in all four series ($p<0.001$ $p<0.01$ $p<0.001$ and $p<0.02$)

It will be seen from Fig 7 that osmolality was lower in series 2 than in series 1 and that there were highly significant differences both in intercepts ($p<0.001$) and in regression coefficients ($p<0.001$) The differences do not change when hypertensives (10 0% of the women) are excluded from series 2 As mentioned earlier series 1 did not include any hypertensives

Fig 7 also shows that the differences between

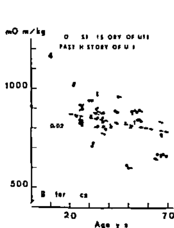


Fig 6 Significant age related decrease in maximal concentrating ability in series 4 (women with bacteriuria/pyuria with and without a past history of UTI) ($r = -0.52$ $p < 0.02$)

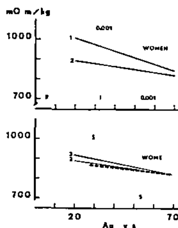


Fig 7 Top Difference in age related decrease in maximal concentrating ability between the female series 1 and 2 (difference in intercepts $p < 0.001$ and regression coefficients $p < 0.001$) *Bottom* Differences between series 2 3 and 4 (not significant)

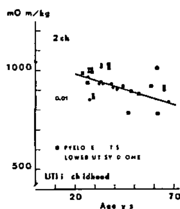


Fig 8 Significant age related decrease in maximal concentrating ability in women in series 2 who had no urinary abnormalities and remembered onset of UTI in childhood before the age of 12–13 ($r = -0.45$ $p < 0.001$)

series 2 3 and 4 were not significant The exclusion of hypertensives from these three series (10 0% 9 2% and 16 2% respectively) does not alter the results The differences between the women in series 3 and series 4 without and those with a past history of UTI were not significant

There were no significant differences between women with no children with 1 child with 2 or 3 children and with 4–12 children

Fig 8 shows maximum concentrating ability of 52 women in series 2 who recalled the onset of upper or lower UTI in childhood before the age of 11–12

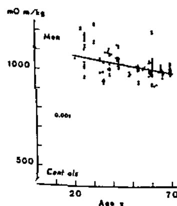


Fig 9 Significant age related decrease in maximal concentrating ability in male controls who had no urinary abnormalities and no past history of UTI ($r = -0.31$ $p < 0.001$)

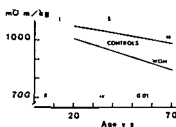


Fig 10 Difference in age related decrease in maximal concentrating ability between female (series 1) and male controls with no urinary abnormalities and no past history of UTI (difference in intercepts not significant in regression coefficients significant $p < 0.01$)

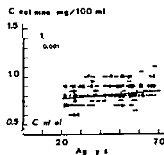


Fig 11 Significant age related increase in serum creatinine concentrations in female series 1 ($r = 0.27$ $p < 0.001$)

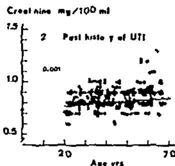


Fig 12 Significant age related increase in serum creatinine concentrations in female series 2 ($r=0\ 28$ $p<0\ 001$)

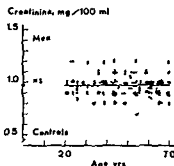


Fig 13 Not significant age related changes in serum creatinine concentrations in male controls ($r=0$ NS)

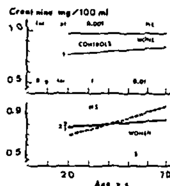


Fig 14 Top Differences in age related changes in serum creatinine concentrations between female series 1 and male controls (significant differences in intercepts $p<0\ 001$ and regression coefficients $p<0\ 01$) Bottom Differences between female series 1 ? 3 and 4 (not significant)

The kidney function was not impaired in these women compared with those who had a later onset of symptoms

There were no significant differences between 51 women in series 2 with a past history of upper UTI and 121 women without such a history

Fig 9 illustrates the individual values for osmolality in relation to age in the male controls Kidney function decreases significantly

Fig 10 shows the higher osmolality in the male than in the female controls the difference in the ... on coefficients was significant ($p<0\ 01$) ... difference in intercepts was not significant

... creatinine concentrations

Figs 11 and 12 show a significant increase in the serum creatinine concentrations with rising age in the female series 1 ($p<0\ 001$) and series 2 ($p<0\ 001$) Fig 14 shows a similar increase in the other female series There are no significant differences between the four series The exclusion of hypertensive cases from series 2–4 did not influence the results The difference was not significant between women with and without a past history of UTI in series 3 and 4

From Fig 13 it will be seen that the serum creatinine concentrations are high and do not decline with rising age in the male controls Fig 14 shows significant differences in intercepts ($p<0\ 001$) and in regression coefficients ($p<0\ 01$) between the male and the female controls

Urea N concentrations in serum

Figs 15 and 16 show the increases in SUN concentration noted at the medical examination of the

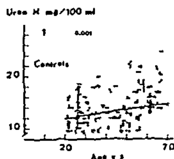


Fig 15 Significant age related increase in serum urea N (SUN) concentrations in female series 1 ($r=0\ 31$ $p<0\ 001$)

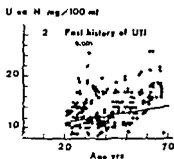


Fig 16 Significant age related increase in SUN concentrations in female series 2 ($r=0\ 38$ $p<0\ 001$)

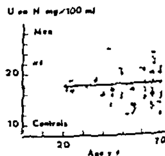


Fig 17 Not significant age-related changes in SUN concentrations in male controls ($r=0$ NS)

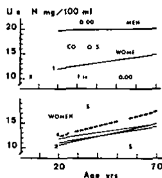


Fig 18 Top Differences in age related changes in SUN concentrations between female series 1 and male controls (significant differences in intercepts $p<0.001$ and in regression coefficients $p<0.001$) *Bottom* Differences between female series 1 2 3 and 4 (not significant)

female series 1 and 2 The corresponding values for the male controls are shown in Fig 17 The increase with age is highly significant ($p<0.001$) in the women but not significant in the men who have higher levels than the women Fig 18 shows highly significant differences in intercepts ($p<0.001$) and regression coefficients ($p<0.001$) between the female and the male controls but non significant differences between the female series 1 2 3 and 4

Findings at intravenous urography

Table I lists the number of persons examined by urography in each of the four series and the presence of duplicity and pyelonephritis The percentages of women and men thus examined are reported under Study Population

Duplicity of pelvis (and ureter) was noted in 5 7 5 0 3 7 and 6 2% respectively in series 1 2 3 and 4 and in 3 8% in the male control series The anomaly was bilateral in 2 cases and unilateral in the others Unilateral duplicity and pyelonephritis were present in 2 cases

Abnormalities suggestive of pyelonephritis (papillary necrosis with or without calcifications scars shrinking) were noted in 1 0 9 9 6 0 9 3% respectively in series 1–4 and in 1 3% of the male controls In 2 hypertensives in whom i v urography showed no abnormalities renal angiography revealed scars in one kidney and in both kidneys respectively The differences between the figures for series 2–4 are not significant The differences between the said series and the female and male controls may be influenced by the selection the controls being normotensive having no urinary abnormalities and no past history of UTI (or urolithiasis)

In the two subgroups within series 3 and 4 women without and with a past history of UTI i v urography showed pyelonephritis in 7 5 and 6 0% respectively in series 3 and in 6 1 and 11 3% respectively in series 4 The latter percentage rose as a result of the afore mentioned angiographic findings in 2 patients with normal urograms and therefore the groups are not fully comparable Furthermore the number of cases within the subgroups is too small to allow conclusions about the differences

In series 2–4 59 women remembered the onset of lower UTI before the age of 12 or 13 years Urography was carried out in 42 (71 2%) of them and revealed pyelonephritis in 2 (4 8%) being unilateral in 1 and bilateral in 1 case Nineteen women remembered having had upper UTI in childhood radiological examination performed in

Table I *Intravenous urography number of women and men examined in each series number and percentage of cases with duplicity of pelvis (and ureter) and abnormalities suggestive of chronic pyelonephritis (interstitial nephritis)*

Radiological findings	Females Series 1 ($n=105$)	Series 2 ($n=121$)	Series 3 ($n=134$)	Series 4 ($n=129$)	Male controls ($n=79$)
Duplicity					
No	6	6	5	8	3
%	5 7	5 0	3 7	6 2	3 8
Pyelonephritis					
Unilateral (no)	1	7	7	10	1
Bilateral (no)	–	5	1	2	–
Total (%)	1 0	9 9	6 0	9 3	1 3

Table II *Intravenous urography percentage of women examined in each series and in each age group percentage of findings of duplicity of pelvis (and ureter) and abnormalities suggestive of chronic pyelonephritis (interstitial nephritis)*

	Age group (%)				
	21–30 (n=85)	31–40 (n=105)	41–50 (n=102)	51–60 (n=104)	61–70 (n=93)
Series					
1 (n=105)	20 0	20 0	20 0	19 0	21 0
2 (n=121)	17 4	25 6	24 8	19 8	12 4
3 (n=134)	14 9	18 7	18 7	24 6	23 1
4 (n=129)	17 8	21 7	20 2	20 9	19 4
Radiological findings (%)					
Duplicity	4 7	3 8	4 9	5 8	6 3
Pyelonephritis	1 2	2 9	5 9	11 5	11 8

15 (78 9%) of them revealed pyelonephritis in 5 (30 0%) being unilateral in 2 and bilateral in 3 cases

In series 2–4 32 women had a past history of UTI and of urolithiasis The co-existence of these two conditions can be explained in part by the fact that urolithiasis might elicit frequency and dysuria Most of these patients however had symptoms of UTI without any associated attacks of pain The concretions passed spontaneously in all of them except 4 who were operated upon In papillary necrosis a detached papilla can have elicited an ... of pain In connection with the medical ex... 30 (93 8%) of the 32 women were sub... to urography The urogram was normal in ... (76 7%) 3 had undergone nephrolithotomy Urography showed pyelonephritis in 7 (23 3%) 1 had undergone operation

Table II shows a fairly uniform distribution of the percentage of cases examined in the different age groups of the female series and an age related continuous increase in the occurrence of pyelonephritis from 1 2 to 11 8%

Hypertension

Sustained hypertension was defined as repeated finding of a BP of 165/105 mmHg or more in new cases or cases diagnosed earlier and undergoing antihypertensive treatment In many of the latter cases the BP readings were still too high because of inadequate dosages of the drugs Of the women in series 2 3 and 4 9 3 7 9 and 9 9% respectively were under treatment at the time of the medical examination As new cases were discovered the proportions increased to 9 9, 8 7 and 16 9% With one exception the hypertensives were over 40 years old 82% being over 50

As mentioned earlier patients with hypertension were excluded from the female and male control series

There was no significant difference between series 2 3 and 4 with respect to data on known hypertension in parents and/or siblings

Hypertension was present in one third of the patients with radiological abnormalities of "pyelonephritis type The figures were too low to allow a comparison between patients with unilateral and bilateral pyelonephritis" or between such cases in series 2 3 and 4

COMMENTS

Selection of material—asymptomatic bacteriuria

Asscher and his group (6 7 8) selected their bacteriuric subjects by screening women who visited the local hospital Their series consisted of non pregnant women in the age range 20–65 years 108 being bacteriurics and 88 controls matched for age civil status and maternity Since 91% of the bacteriurics and 62 5% of the controls had a past history of UTI Asscher concluded that "asymptomatic bacteriuria is not truly asymptomatic More important these findings show that screening for significant bacteriuria often fails to detect urinary infection at an early stage "

However with such a basis for comparisons any analysis of the importance of a past history of UTI

seems to be unreliable This holds good especially for the modern meaning of the term civil status Our results show that asymptomatic bacteriuria is prevalent 40% of the asymptomatic bacteriuric non pregnant women had no past history of UTI

Urea N concentration in serum A total of 844 asymptomatic non pregnant women aged 21–70 were divided into four series 1) Controls with no urinary abnormalities and no history of UTI 2) Women with no urinary abnormalities and a past history of UTI 3) Women with sterile pyuria 4) Women with bacteriuria/pyuria Each of the latter two series comprised two subgroups namely women who had no past history of UTI (about 40%) and women who had such a history (about 60%) There was no significant difference in the SUN level between the four female series or between the subgroups The exclusion of hypertensives did not influence the results The levels of SUN increased similarly with increasing age in all the series

These results agree in the main with previously published series selected by other methods which have been summarized by Asscher (7) and Freedman (12)

However in our series of 232 male controls the level of SUN was significantly higher than in the female controls and did not rise with increasing age

The age related increase of urea in women has been attributed to ageing (6 8) In women however a UTI symptomatic or asymptomatic is more common than in men and might possibly be a causal factor in this ageing in women In the present population study by means of a questionnaire we found a past history of symptomatic upper UTI in around 1% and of lower UTI in 3% of the men in the age range 31–69 years In the women aged 21–70 the incidence of symptomatic upper UTI rose with increasing age from about 5% to 14% whereas the figures for lower UTI remained fairly constant in all the age groups about 15–17%

The fact that none of the bacteriuric women in Asscher s series had a blood urea concentration in excess of 55 mg/100 ml led to the following conclusion This seems surprising if bacteriuria in the adult is in fact an important cause of progressive kidney failure According to clinical experience a sufficiently large material representing a real cross section of the total population should have yielded findings of raised BUN levels (10) As mentioned in the foregoing our material does not represent a cross section of the total population

Summarizing the results of determinations of the SUN level in women and men do not seem to justify the quoted categorical conclusion by Asscher and Freedman that UTI does not injure the kidneys

Finally according to our results elimination of the bacteriuria by treatment did not cause any significant alteration in the SUN level in series 4

Serum creatinine concentration Determination of creatinine in serum does not seem to have been employed in earlier population studies Our results in the female series 1 2 3 and 4 and in the male control series agreed with those obtained for the SUN level The exclusion of hypertensive cases did not influence the results The values rose with increasing age in the female series The level in the male controls was significantly higher than that in the female controls and did not rise with age Accordingly our comments on the results relating to SUN are also valid for the results obtained by this method Eradication of the infection did not change the creatinine level in the bacteriuric series

Maximal concentrating ability In the female series 1 (controls with no urinary abnormalities and no past history of UTI) the level of osmolality was significantly higher than in series 2 (women with no urinary abnormalities who had had UTI in the past) The highly significant differences were not influenced by the exclusion of hypertensives from the latter series There were no significant differences between series 2 and either series 3 (women with sterile pyuria 60% had a past history of UTI) or series 4 (women with bacteriuria/pyuria 60% had a past history of UTI) There was no significant difference between the subgroups of women without and with a past history of UTI in series 3 and series 4 respectively or between the last mentioned subgroups and series 2 Women in these subgroups who had not had UTI in the past had significantly lower osmolality than the female controls (series 1)

Consequently there seems to be a cause and effect relationship between reduced maximal concentrating ability and factors such as abnormal urinary findings in the form of sterile pyuria or bacteriuria/pyuria a past history of UTI and a combination of these factors

Asscher and collaborators found a significant impairment of the concentrating ability among those women who remembered an onset of symptoms of UTI from childhood Among the women in our series 2 the concentrating ability was not significantly impaired among those who remembered the

onset of UTI in childhood (before the age of 12–13) compared with those who had noticed a later onset of symptoms Cases with onset of UTI in the first few years of life could not be studied because of few and uncertain data There was no significant difference between women with and without a past history of symptoms of upper UTI in childhood

In all the series the concentrating ability decreased with rising age The greatest age related reduction was observed in women who had no urinary abnormalities and a past history of UTI (series 2) and in pyurics and bacteriurics/pyurics with or without a past history of UTI (series 3 and 4) there were no significant differences between these three series when compared with one another

The male controls had a higher concentrating ability than the female controls The difference in the regression coefficients was highly significant the difference in the intercepts was not significant

Referring to the discussion presented here it should be stressed that hypothetically not only symptomatic but also asymptomatic UTI may affect renal function in women and provide an essential cause of ageing , as revealed by these methods Of course other possible contributory factors must be taken into consideration Maternity seems not to be a factor of significant importance

Consequently in comparison with the SUN and the creatinine concentration tests determination of axima! concentrating ability seems to be the thod of choice in population studies of this kind

n earlier report (2) showed that elimination of e infection by mostly long term treatment led to statistical normalization of the defective concentrating ability in a series of 102 non pregnant bacteriuric women Ninety nine of these cases are included in the present enlarged series The therapeutic result was similar (3) Our results agree with those obtained by treatment of bacteriuric pregnant women in whom the defective concentrating ability was almost completely reversed by elimination of the infection (14)

Unfortunately a systematic study on long term treatment of our series of women who had sterile pyuria was not possible because of insufficient resources An interesting finding in this connection is that sterile pyuria can be cleared or reduced by antibacterial treatment (11)

Two questions that remain unanswered are to what extent such factors lead to progressive kidney damage and how effectively adequate treatment of the urinary tract infection will prevent this development in non pregnant women who do not suffer from uropathy, urolithiasis, or other abnormalities that predispose to infection

Radiological abnormalities suggestive of chronic pyelonephritis No other study seems to have compared series of bacteriuric non pregnant women with series other than non bacteriuric controls with regard to acquired abnormalities of pyelonephritis type The figures for our series 2 3 and 4 were 10 9, 7 3 and 8 5% Thus it seems impossible to conclude that bacteriuria per se would necessarily bear any relationship to the appearance of radiographic abnormalities of this type unless it is assumed that the women in these series had antecedent bacteriuria

Of the patients with chronic pyelonephritis" about one third had hypertension a past history of urolithiasis or an onset of upper UTI in childhood Patients with a past history of urolithiasis had also had UTI in the past Since such cases were excluded from the female and the male control series the figures for the incidence of pyelonephritis" in the controls (1 0% and 1 5%) cannot be compared with those for the rest of the female series

A comparison does not seem possible between our findings and those of others e g Asscher et al (6 8) who reported a series of bacteriuric non-pregnant women including cases with obstructive uropathy and stones conditions that predispose to infection and influence the results of antibacterial treatment I v urography showed abnormalities in about one third of 93 cases thus examined Twelve had scars and 4 also had stones and 2 of these hydroureter hydronephrosis as well another 4 women had stones 2 had hydroureter hydronephrosis and 6 had hydroureter Of 50 examined controls 1 had scars , 1 hydroureter 1 difference in size and 1 polycystic kidneys

The present material shows an age related continuous increase in the occurrence of abnormalities of the pyelonephritis type in the female series from 1 2 to 11 8% This seems not to agree with the assumption that this kind of kidney damage originates mainly in childhood (6) The percentage of congenital abnormalities (duplicity) was fairly uniform in all age groups

The results of a preliminary follow up of individuals included in our study up to the middle of 1974 have been published (2) This follow up could be extended and continued up to Sept 1975 (3)

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The Diagnostic Value of Protein Clearances in Rejection of Human Renal Allografts

L Hemmingsen H Jensen P Jest and P Skaarup

From Medical Department P Division of Nephrology Rigshospitalet University Hospital Copenhagen the Departments of Nephrology and Thoracic Surgery Odense University Hospital Odense and the Department of Clinical Chemistry Central Hospital Nykøbing Falster Denmark

ABSTRACT The relative clearances of transferrin, haptoglobin, IgG and IgA were used as a diagnostic test for detection of acute rejection episodes after renal transplantation, using an automated immunoprecipitin reaction Thirty six out of 40 rejections were predicted by 0–5 days The sensitivity and specificity were 90% and 98%, respectively The predictive value of a positive diagnostic test was 86% and of a negative 99% False positives caused by fever and urinary obstruction could probably be excluded by the finding of an increased urinary excretion of β_2-microglobulin prior to the increased relative clearance of transferrin haptoglobin, IgG and IgA This would improve the predictive value of a positive test to 95% Daily or more frequent protein clearance determinations should be performed in renal transplant patients, since the method used was easy, rapid and suited for sequential analysis, and the procedure was without risk for the patient

Increased urinary protein excretion in connection with rejection episodes following renal transplantation is reported in several papers (2 4 13 15 18) The protein determinations in these studies were performed by time consuming methods Consequently a prediction of rejection could not be performed

In order to surmount this difficulty we introduced an automated immunoprecipitin reaction for the protein determinations in the urine from renal transplant patients (10) In this investigation 11 out of 13 rejection episodes could be predicted by 1–5 days when the renal clearance of 10 different proteins was examined Estimations of the sensitivity and specificity revealed that the clearances of transferrin haptoglobin IgG and IgA are to be preferred as a diagnostic test for detection of acute rejection episodes

Therefore the aim of the present study was to use the clearances of transferrin haptoglobin IgG and IgA as a diagnostic test for acute rejection episodes and to assess the predictive value of a positive and a negative diagnostic test respectively Further we examined the protein clearances in clinical events which may interfere with the signs of acute rejection e g fever caused by various infections and urinary obstruction caused by urinary extravasation lymphoceles or large perigraft abscesses (3 19 20) For this purpose we also studied the urinary excretion of β_2 microglobulin because events other than rejection episodes may be accompanied by a tubular type of proteinuria (5 6 9 10 22)

The proteins were measured as protein related material since some of them could be present in the urine either as whole molecules split products or aggregates with preserved antigenic determinants (14 21 25)

Reprint requests to Dr P Skaarup Department of Urology Hvidovre Hospital DK 2650 Hvidovre Denmark

METHODS

Protein analysis

Collections of consecutive 24-hour urines were started immediately after renal transplantation in 39 patients and on the 6th–11th day after transplantation in 15 patients The periods of examination lasted for 5–112 days During the first postoperative days the urine was collected via an

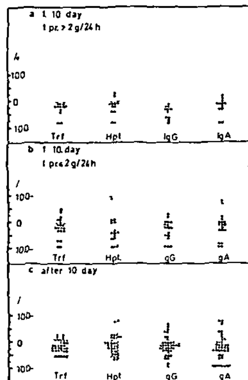


Fig 1 Day to-day variation of the relative clearances of transferrin haptoglobin IgG and IgA in four patients serving as references Patients with a total urinary protein excretion >2 g/24 h demonstrated during the first 10 post transplant days only few positive day to-day variations with an upper empiric limit of +25% (*a*) Patients with a total urinary protein excretion <2 g/24 h showed during the first 10 posttransplant days positive day to-day varia ns with an upper empiric limit of +100% (*b*) After the posttransplant day the positive day to-day variations onstrated an empiric upper limit of +100% (c) None f the observations above the upper limits occurred simultaneously

indwelling ureteric catheter Venous blood was drawn every second or third day The urine and serum samples were kept below +4 C without addition of preservatives and the protein analyses were carried out within 5 days of collection

The determination of specific proteins in urine and serum was performed on an AutoAnalyzer (Technicon Corp Tarrytown NY) The method used was an im munoprecipitin reaction (AIP) The precipitate was meas ured by fluoronephelometry In order to increase the sensitivity the standard method was modified as de scribed previously (7 11) The average sensitivity was 0 1 mg/l depending on the range of measurement The preci sion of the method (coefficient of variation) expressed as daily within run and between runs averaged 4 5% and 9 5%, respectively The analytical capacity was 60 speci mens per hour

The urinary total protein concentration was determined by means of a tetrabromphenolblue reaction (8) The uri

Table I *Criteria for considering an increased rela tive protein clearance significant*

Days after renal trans plantation	Degree of pro- teinuria (g/24 h)	Criteria
1–10	>2	An increase exceeding the relative protein clear ance on the previous day by at least 25%
1–10	≤2	An increase exceeding the relative protein clear ance on the previous day by at least 100%
>10	–	An increase exceeding the relative protein clear ance of the stabilized level by at least 100%

nary and serum creatinine concentrations were de termined by Jaffe s reaction (AutoAnalyzer) The pH of all urine samples was above 5 5 Blood contamination was estimated semiquantitatively by Sangur®

Calculation principles

The relative clearance of the high molecular weight pro teins transferrin haptoglobin IgG and IgA was calcu lated by the formula $(U_P \ V/S_P)/(U_{Cr} \ V/S_{Cr})$ and the rela tive excretion of β_2-microglobulin by the formula $(U_P \ V)/(U_{Cr} \ V/S_{Cr})$ where U_P and S_P denote the urinary and serum concentration of the specific protein U_{Cr} and S_{Cr} the urinary and serum concentration of creatinine and V the urine volume per unit of time

The day to-day variation of the relative clearances of transferrin haptoglobin IgG and IgA was expressed as the percentage differences between two days calculated in proportion to the first day

The calculations of the sensitivity and specificity and the predictive value of a positive and negative diagnostic test were performed as described by Vecchio (24) and Wulff (26) Positive or negative diagnostic tests were indi cated by the presence or absence of significantly increased relative protein clearances respectively Consequently true positives (TP) were positive tests in the presence of rejection false positives (FP) were positive tests in the absence of rejection true negatives (TN) were negative tests in the absence of rejection and false negatives (FN) were negative tests in the presence of rejection

STUDY POPULATION

Reference cases

Four patients running a posttransplant course without any complications served as references These patients have been presented in a previous paper (10)

Fig 1 demonstrates 79 day to-day variations of the rela tive protein clearances in these four patients During the first 10 posttransplant days macroscopic or microscopic

Table II *Prediction of rejection compared with the clinical diagnosis of rejection*

	Rejection	No rejection	Total
Prediction of rejection	36	6	42
No prediction of rejection	4	363	367
Total	40	369	409

haematuria was always present although in gradually decreasing amounts and was probably the main source of urinary protein content Consequently the reference cases were divided at the 10th posttransplant day When blood contamination was severe and total urinary protein excretion >2 g/24 h almost all day to-day variations of the relative protein clearances turned out negative and no positive day to-day variations exceeded 25% (Fig 1*a*) On the other hand when blood contamination was moderate with a total urinary protein excretion ≤2 g/24 h positive day to-day variations were more frequent but exceeded 100% in only one case (Fig 1*b*)

After the 10th posttransplant day when haematuria had disappeared the total urinary protein excretion stabilized on a lower level the day to-day variations of the relative protein clearances ranging from −100% to about +100% (Fig 1*c*)

The criteria for considering an increased protein clearance significant were based on the findings of the day to-day variations in the reference cases and are listed in Table I

Prediction of rejection was defined as a significantly increased relative clearance of at least 2 high molecular weight proteins (10)

Patients

The study concerned 50 renal transplant patients 19 women and 31 men aged 5–63 years Twenty of them have been studied earlier (10) The posttransplant immunosuppressive regimen consisted of azathioprine and prednisone Acute rejection episodes were diagnosed by conventional means as described previously (10 17) and treated with methylprednisolone or increased doses of prednisone The day of rejection was defined as the day when antirejection therapy started if not disproved subsequently by a definite histological examination

Acute renal failure complicated the course in 10 patients who were treated with haemodialysis for 1–2 weeks after transplantation Five patients had urinary obstruction due to lymphoceles in 2 large perigraft abscesses in 2 and necrosis of ureter with urinary extravasation in 1 Six patients had severe infections i e perigraft abscesses lung abscesses pneumonia and sepsis

RESULTS

Predictive value

A clinical diagnosis of rejection was made 40 times in 37 patients In 6 patients rejection occurred before the observation period and no rejection episodes were diagnosed in 7 patients Prediction of rejection was made without any information about the clinical diagnosis of rejection

Table II shows the predictions of rejection compared with the observed rejection episodes Among 42 predictions antirejection therapy was given in 36 cases (TP) and no therapy was given in 6 cases (FP) In 367 situations where no predictions were made antirejection therapy was given in 4 cases (FN) and no therapy was given in 363 cases (TN)

The sensitivity defined as the probability that the relative protein clearance is significantly increased in connection with acute rejection episodes was

$$\frac{TP}{TP+FN}=\frac{36}{36+4}=90\%$$

The specificity defined as the probability that the relative protein clearance is not increased significantly in courses without rejections was

$$\frac{TN}{TN+FP}=\frac{363}{363+6}=98\%$$

The predictive value of a positive test defined as the probability that acute rejection episodes do occur when the relative protein clearances are increased significantly was

$$\frac{TP}{TP+FP}=\frac{36}{36+6}=86\%$$

The predictive value of a negative test defined as the probability that rejection does not occur when the relative protein clearance is not increased significantly was

$$\frac{TN}{TN+FN}=\frac{363}{363+4}=99\%$$

True positives

Table III shows the basis for prediction of 36 rejection episodes by clearance determinations of transferrin haptoglobin IgG and IgA For each prediction the significant increase of the relative protein clearances is indicated by the day to-day variation Twenty five rejections were predicted 1–5 days before and 11 rejections at the same time as antirejection therapy was started Rejection was predicted by 5 days in 1 patient (no 2) and by 4 days in 2 patients (nos 4 and 5) with acute renal failure Antirejection therapy was followed by a decrease in

Table III *Prediction of 36 acute rejection episodes in 33 patients in relation to posttransplant time to al urinary protein excretion and occurrence of acute renal failure*

Pat no	Total urinary protein (g/24 h)	Day of prediction	Days between prediction and antirejection therapy	Increase of relative protein clearances (%)[b] Trans ferrin	Haptoglobin	IgG	IgA
1	1 0	77	5	180	–	–	110
2[a]	1 5	2	5	280	810	450	510
3	10 1	3	5	720	730	560	410
4[a]	0 4	10	4	150	–	130	110
5	0 7	10	4	100	–	200	–
6	2 0	5	4	230	–	100	110
7	0 2	6	4	–	190	140	110
8	0 4	11	4	220	310	230	150
9	0 5	20	3	150	–	140	–
10	1 0	6	3	–	110	–	120
11	2 1	8	2	130	–	130	420
12	0 4	5	2	–	360	–	230
7	3 0	32	2	770	420	690	500
13	0 5	34	2	260	–	100	370
14	0 7	5	2	–	–	190	140
15	2 9	5	2	60	70	80	70
16	1 3	6	1	110	100	–	–
17	2 2	3	1	42	94	34	47
18	0 7	6	1	–	870	–	140
19	3 1	7	1	260	410	300	350
20	1 9	28	1	230	–	200	130
21	1 0	7	1	120	–	100	100
13	1 5	6	1	580	680	690	550
22	1 5	9	1	120	160	130	–
23	1 1	7	1	540	520	630	340
24	0 4	40	0	2 810	–	–	2 680
25	0 9	5	0	490	2 450	2 780	37 500
11	1 5	33	0	230	–	210	–
26	0 7	3	0	–	300	–	220
7	0 1	6	0	490	1 540	600	620
	0 5	7	0	–	–	120	120
	0 9	5	0	100	450	190	–
	1 1	5	0	280	220	380	500
1	1 2	6	0	–	430	100	170
32	1 3	13	0	340	200	360	160
33	3 1	28	0	110	1 070	480	100

[a] Patients with acute renal failure
[b] *Indicated for each prediction when the increase was significant*

the relative protein clearances in all but 6 patients with irreversible rejections In these patients (nos 3, 18 25 27 29 and 30) the relative protein clearance continued to increase in spite of antirejection therapy

False positives

Six predictions were made without subsequent antirejection therapy being started One prediction was made when a simultaneous suspicion of rejection was claimed in 4 out of 7 renograms On 3 occasions rejection was predicted in the presence of fever (>38 5 C) caused by a perigraft abscess and pulmonary abscesses with sepsis Rejection was predicted in 1 patient with urinary obstruction due to a lymphocele In these cases the relative excretion of β_2 microglobulin increased prior to the excretion of the other proteins In 1 patient no cause of the false prediction could be observed

True negatives

The courses without rejection episodes were characterized by extremely high relative protein clearances immediately after renal transplantation followed by gradually decreasing clearances during the first 10 posttransplant days After the 10th day

only minor changes in the protein excretion occurred. Seven patients with acute renal failure were included in the true negatives.

False negatives

Four rejection episodes were not predicted. In 1 patient with an accelerated irreversible rejection on the 2nd posttransplant day the relative clearance of all 4 proteins increased significantly on the 3rd day after transplantation. In 1 patient the creatinine clearance decreased less than 5 ml/min and in 2 patients no decrease of the creatinine clearance was observed before or during the institution of antirejection therapy.

Excretion of β_2-microglobulin

A persistently increased β_2 microglobulin excretion preceded the increased clearance of the high molecular weight proteins by 1–2 days in 6 patients with fever caused by pneumonia, pulmonary abscesses, perigraft abscess and sepsis, and by 6–11 days in 5 patients with urinary obstruction due to urinary extravasation, large perigraft abscesses and lymphoceles. In none of these cases did the creatinine clearance decrease.

DISCUSSION

A prerequisite for evaluating a diagnostic test is the future certainty of the true diagnosis based on a definite histological examination or the subsequent clinical course (26).

The diagnosis of rejection was based on histological examination of the removed transplant in 11 cases and evidenced by histological examination of percutaneous graft biopsies in 2 cases. The subsequent clinical course was no reliable confirmation of the diagnosis of rejection, since it was modified by the antirejection therapy (20). Therefore the institution of antirejection therapy was used as a marker of rejection.

This adaptation involves some disadvantages, since rejection might be present when no antirejection therapy was started, and antirejection therapy might be started in the absence of rejection. Some of the false positives and false negatives might be attributed to these possibilities.

In the differential diagnosis between acute rejection and acute renal failure the protein clearance determinations seemed to be of clinical value, since rejections could be predicted several days ahead without false positives or false negatives.

Characterization of increased urinary protein excretion in connection with febrile diseases and urinary obstruction has been reported in several papers (12, 16, 22). The false positives caused by an increased relative clearance of the high molecular weight proteins in relation to fever or urinary obstruction were preceded by a persistently increased relative excretion of β_2 microglobulin. If corrected for these events, using β_2 microglobulin or another low molecular weight protein as marker, the false positives could be reduced from 6 to 2, thus improving the predictive value of a positive diagnostic test from 86% to 95%. The increased excretion of β_2 microglobulin in febrile cases might be caused either by an increased serum concentration or by a decreased tubular reabsorption (12, 16). The increased β_2 microglobulin excretion in urinary obstruction was probably caused by impaired tubular reabsorption, since the creatinine clearance was unchanged in these cases. Tubular ischaemia is reported to be an etiological factor (1, 23).

In conclusion, acute rejection episodes in renal transplant patients monitored with the AIP system were predicted 0–5 days in advance. The predictive value of a positive test was 85% if predictions were performed without regard to the relative excretion of β_2 microglobulin. When the determination of the relative β_2 microglobulin excretion was included in the definition of prediction, the predictive value of a positive test was 95%.

On these conditions we propose daily protein clearance determinations in renal transplant patients. If rejection is suspected, more frequent determinations should be performed.

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Comparative Natriuretic and Diuretic Efficacy of Theophylline Ethylenediamine and of Bendroflumethiazide during Long-Term Treatment with the Potent Diuretic Bumetanide

Permutation Trial Tests in Patients with Congestive Heart Failure

Bjarne Sigurd and Knud H Olesen

From Medical Department B Rigshospitalet Copenhagen Denmark

ABSTRACT The additive natriuretic and diuretic effects of theophylline ethylenediamine and of bendroflumethiazide have been compared in permutation trial tests in patients with advanced congestive heart failure receiving long term treatment with the highly potent diuretic, bumetanide Statistical analysis of renal water and electrolyte excretion revealed that theophylline ethylenediamine, 400 mg orally, and bendroflumethiazide, 5 mg orally, had very similar effects, both quantitatively and qualitatively The mechanism of action of the supplementary diuretics is discussed It is concluded that theophylline ethylenediamine represents a useful alternative to thiazide diuretics when supplementary natriuretic treatment is considered in patients with congestive heart failure during long term treatment with potent diuretics The significance of maintaining the potassium balance during such a combined regimen is stressed

In patients with advanced congestive heart failure a combination of diuretics is often required for adequate control of sodium and water retention In a previous communication we have described the additive natriuretic effect of a single oral dose of theophylline ethylenediamine and bumetanide in patients with congestive heart failure receiving long term treatment with bumetanide (17) Similarly the additive natriuretic effect of a single dose of bendroflumethiazide and bumetanide or furosemide during long term treatment with one of the latter potent diuretics has been reported (14 18) Bumetanide is a new highly potent diuretic which is very similar to furosemide in terms of renal tubular action and is equipotent with this diuretic in a weight ratio of 1 40 (15)

The objectives of this report are to compare the natriuretic efficacy of theophylline ethylenediamine and of bendroflumethiazide in patients with congestive heart failure receiving long term treatment with bumetanide to analyse the qualitative effects of these drugs when used as supplementary natriuretics in the above setting and to discuss their mechanisms of action and their side-effects

The present study comprises three permutation trial tests in patients with advanced congestive heart failure The first two trials are conducted in patients receiving long term treatment with bumetanide while the third trial is performed in patients who had not previously received potent diuretics

METHODS

Patient selection

Eighteen adult subjects 11 men and 7 women with organic heart disease and signs of congestive heart failure requiring relatively intensive diuretic treatment were selected for study Their mean age was 54 3 years The clinical diagnoses were rheumatic valvular heart disease in ten patients ischaemic heart disease in six and cardiomyopathy in two

Twelve patients had received long term treatment with the potent diuretic bumetanide for more than two weeks in a dosage of 4 mg/day (2 mg b i d)

Permutation trial tests

Since the response to diuretic treatment varies not only with the drugs used but also with the pathophysiologic status of the patients and with the sequence of administra

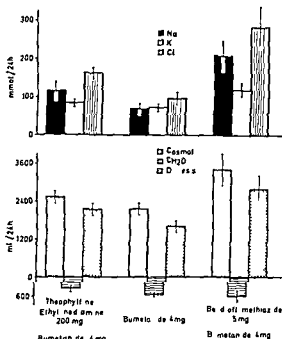


Fig 1 Mean values for urinary electrolyte and water excretion in trial I in relation to diuretic treatments Vertical bars indicate ± S E M

tion of drugs the study was designed to minimize the effects of the latter variables It took the form of permutation trial tests in which the drug treatment followed the rotation scheme shown in Table I This type of programme ensures that within each trial all treatments are to each patient and that each treatment has an chance of being used on the first second or third of the trial (4 14 17 18) A random allocation of patients to treatment programmes was secured

During the study the patients received digoxin as before a 3 g sodium chloride diet a supplement of 3 g potassium chloride daily and 1 500 ml fluid per day During the hospital stay the patients were up and about in the day time Body weight serum creatinine osmolality and electrolytes were determined every morning 24-hour urines were collected at 7 a m for assay of Na K Cl creatinine and osmolality Daily creatinine clearance osmolal clearance and free water excretion were calculated as previously described (15) With their informed consent the patients were studied as follows

Trial I involved 6 patients receiving digoxin bumetanide 4 mg (2 mg b i d) and supplementary potassium chloride (45 mmol/day) A comparison was made of the effects of supplementary theophylline ethylenediamine 200 mg as a single oral dose in the morning (*A*) of placebo (*B*) and of supplementary bendroflumethiazide 5 mg as a single oral dose in the morning (*C*)

Trial II concerned 6 patients receiving the same treatment as patients in trial I A comparison was made of the effects of supplementary theophylline ethylenediamine 400 mg (200 mg orally b i d) (*A*) of placebo (*B*) and of supplementary bendroflumethiazide 5 mg orally in the morning (*C*)

Trial III covered 6 patients receiving digoxin and supplementary potassium chloride (45 mmol/day) without bumetanide medication A comparison was made of the effects of theophylline ethylenediamine 400 mg (200 mg orally b i d) (*A*) of bendroflumethiazide 10 mg (5 mg b i d) (*B*) and of the combination of theophylline ethylenediamine 400 mg and bendroflumethiazide 10 mg (*C*)

Statistical methods

The design of the experiments permitted an evaluation of drug effects separately from the influence of different patients and of varying sequence of administration of drugs The quantitative effects were analysed by means of the Wilcoxon test for pair differences (2) In the case of qualitative effects interrelations between outputs of electrolytes and water following various treatments were compared in a covariance analysis (19)

Terminology

Dose addition refers to the combined effects of two drugs acting on the same receptors where doses of one drug are able to substitute for those of the other in proportion to their relative potency Deviations from dose addition are termed supra additive or infra additive and usually imply that the drugs act by different mechanisms

Effect addition or summation refers to the combined effects of two drugs acting through different mechanisms when the responses are equal to the sum of their individual effects Deviations from effect addition are usually termed supra additive or infra additive (3)

RESULTS

Studies during Long Term Treatment with Bumetanide

Quantitative effects of supplementary diuretics

Trial I compared the effects of theophylline ethylenediamine 200 mg +bumetanide 4 mg (*A*) of placebo+bumetanide 4 mg (*B*) and of bendroflumethiazide 5 mg +bumetanide, 4 mg (*C*) The

Table I *Sequence of diuretic treatment*

For explanation of A B and C see text

Day of treatment		
1	2	3
A	B	C
A	C	B
B	A	C
B	C	A
C	A	B
C	B	A

Table II *Statistical analysis of renal electrolyte water and solute excretion and of weight loss in trials I II and III (mean 24 hour values ±S E M)*

Urinary excretion	Treatment A	Treatment B	Treatment C	Statistical significance of differences A–B	A–C
Trial I					
Sodium (mmol/24 h)	115±26	68±16	210±40	*	*
Potassium (mmol/24 h)	83±9	72±9	120±18	*	*
Chloride (mmol/24 h)	162±17	96±17	284±55		*
Diuresis (ml/24 h)	2 157±200	1 600±175	2 808±44	*	*
Osmolal clearance (ml/24 h)	2 530±212	2 176±194	3 416±527	*	ns
Free water clearance (ml/24 h)	−373±109	−559±70	−608±171	ns	ns
Creatinine (mmol/24 h)	9 08±1 10	10 38±1 53	9 96±2 06	ns	ns
Weight loss (kg/24 h)	−0 93±0 17	0 15±0 30	−1 05±0 41	*	ns
Creatinine clearance (ml/min)	60±9	66±10	53±7	ns	ns
Trial II					
Sodium (mmol/24 h)	105±14	40±6	159±26	*	ns
Potassium (mmol/24 h)	78±14	71±8	121±22	ns	ns
Chloride (mmol/24 h)	159±36	64±27	183±59	*	ns
Diuresis (ml/24 h)	2 055±207	1 329±173	2 273±444	*	ns
Osmolal clearance (ml/24 h)	2 641±177	1 947±219	2 840±492	*	ns
Free water clearance (ml/24 h)	−586±107	−618±142	−567±156	ns	ns
Creatinine (mmol/24 h)	7 28±1 05	6 70±1 40	7 20±1 39	ns	ns
Weight loss (kg/24 h)	−0 48±0 34	0 02±0 23	−0 65±0 32	ns	ns
Creatinine clearance (ml/min)	51±8	52±12	51±12	ns	ns
Trial III					
Sodium (mmol/24 h)	77±13	54±14	116±13	ns	*
Potassium (mmol/24 h)	99±14	87±13	104±11	ns	ns
Chloride (mmol/24 h)	100±25	94±17	161±18	ns	*
Diuresis (ml/24 h)	1 217±120	942±146	1 404±98	*	*
Osmolal clearance (ml/24 h)	−2 475±284	1 614±329	2 557±242	ns	ns
Free water clearance (ml/24 h)	−1 258±216	−672±346	−1 154±192	ns	ns
Creatinine (mmol/24 h)	10 92±1 90	9 97±1 74	9 38±1 25	ns	ns
Weight loss (kg/24 h)	0 00±0 12	−0 43±0 12	−0 18±0 24	*	ns
Creatinine clearance (ml/min)	80±12	74±12	68±6	ns	ns

ns=not significant (*p*>0 05) **p*<0 05

results are shown with statistical analysis in Table II and Fig 1

Comparison A B The mean values for urinary excretion of sodium potassium chloride water and osmolal clearance were significantly higher after treatment A than after treatment B (*p*<0 05)

Comparison A C The mean values for sodium potassium chloride and water outputs were significantly higher after treatment C than after treatment A (*p*<0 05) Body weight and creatinine clearance were unaffected

Trial II compared the effects of theophylline ethylenediamine 400 mg +bumetanide 4 mg (*A*) of placebo+bumetanide 4 mg (*B*) and of bendroflumethiazide 5 mg +bumetanide 4 mg (*C*) The results are shown with statistical analysis in Table II and Fig 2

Comparison A B The mean values for urinary outputs of sodium chloride water and osmolal clearance were significantly higher after treatment A than after treatment B (*p*<0 05)

Comparison A-C As shown in Table III no significant differences were obtained between urinary excretion of electrolytes water or weight loss although all values tended to be higher after treatment C than after treatment A Creatinine clearance was unaffected during all treatments

Qualitative effects of supplementary diuretics

For the total group of 12 patients participating in trials I and II the interrelations between the 24-hour urinary outputs of Na K Cl Na+K and water on the day of treatment with theophylline ethylene

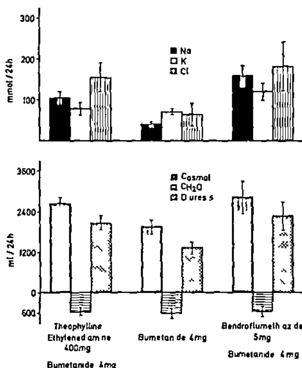


Fig 2 Mean values for urinary electrolyte and water excretion in trial II in relation to diuretic treatments Vertical bars indicate ±S E M

diamine and on the day of treatment with bendroflumethiazide were compared in a covariance analysis (Table III) The statistical comparison of the gressions of K on Na of Cl on Na of H_2O on Na H_2O on Cl or of Na+K on Cl revealed no signifi t differences in variances in slopes or in adjusted means Apparently this type of analysis reveals no significant differences between the two supplementary drugs in terms of renal tubular action in the setting of patients receiving long term treatment with bumetanide

Studies without Bumetanide Medication

Trial III compared the effects of theophylline ethylenediamine, 400 mg (*A*) of bendroflumethiazide 10 mg (*B*) and of theophylline ethylenediamine 400 mg + bendroflumethiazide 10 mg (*C*) The results are shown with statistical analysis in Table II and in Fig 3

Comparison A–B No significant differences were observed in terms of urinary electrolyte or water excretion body weight or creatinine clearance

Comparison A–C Urinary outputs of sodium chloride and water were significantly higher after treatment *C* than after treatment A In terms of sodium and chloride excretion the sum of the effects of both drugs is close to the sum of their individual effects

With regard to the qualitative effects the interrelations between Na K Cl H_2O and Na+K during treatment A and treatment B were compared in a covariance analysis No significant differences were observed in terms of variances slopes or adjusted means

These findings are compatible with the concept that theophylline ethylenediamine and bendroflumethiazide may act on the same renal tubular receptors However they do not exclude an effect addition of two drugs acting at different sites in the nephron (3)

DISCUSSION

The results confirm and extend our previous experience of additive natriuretic and diuretic effects of 1) theophylline ethylenediamine and bumetanide and 2) bendroflumethiazide and bumetanide

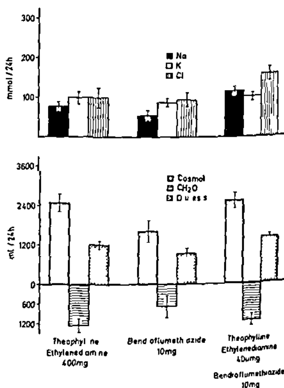


Fig 3 Mean values for urinary electrolyte and water excretion in trial III in relation to diuretic treatment Vertical bars indicate ±S E M

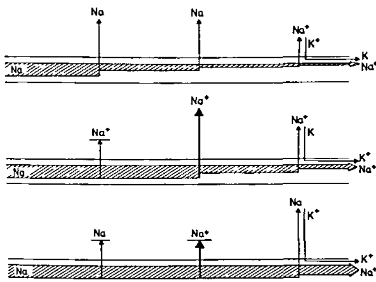


Fig 4 Diagram of the combined effects of diuretics upon renal tubular sodium transport

in patients with congestive heart failure receiving long term treatment with the latter drug (17 18)

In terms of quantitative effects of the supplementary drugs in this setting theophylline ethylenediamine 200 mg as a single oral dose proved less effective than bendroflumethiazide 5 mg as a single oral dose In the comparison of theophylline ethylenediamine 400 mg (200 mg b i d) and bendroflumethiazide 5 mg as a single oral dose no significant differences were found although the urinary outputs tended to be higher after the latter drug

With regard to the qualitative actions of the supplementary drugs no significant differences occurred in covariance analyses of the regressions of K on Na Cl on Na H_2O on Na H_2O on Cl or (Na+K) on Cl Apparently the water and electrolyte excretory patterns after theophylline ethylenediamine and after bendroflumethiazide are strikingly similar as previously stressed by other investigators (9 11 12 16)

It is pertinent to point out that the major and probably the only natriuretic action of theophylline ethylenediamine in this setting should be related to the renal tubular action of this drug In normal man theophylline ethylenediamine may cause a transient increase in renal blood flow and glomerular filtration rate However in patients with congestive heart failure the changes observed in renal haemodynamics and glomerular filtration rate after theophylline ethylenediamine have been very small and inconsistent suggesting that the major action of the drug in this setting is caused by a depression of renal tubular sodium reabsorption (1) Accordingly in the present study creatinine clearance showed no significant change after theophylline ethylenediamine although the oral administration of this drug 400 mg (200 mg b i d) should ensure sufficiently elevated blood or plasma levels to promote a natriuretic action (4 5 6 8 10 21)

In terms of depression of renal tubular sodium re

Table III *Interrelations between urinary Na K Cl and water excretion*

Treatment groups	Measurement *Y*	Measurement *X*
Long term treatment with bumetanide 4 mg (+bendroflumethiazide 5 mg +theophylline ethylenediamine 200–400 mg) (12 pats)	K	Na
	K	Na
	Cl	Na
	Cl	Na
	H_2O	Na
	H_2O	Na
	H_2O	Cl
	H_2O	Cl
	Na+K	Cl
	Na+K	Cl
Not treated with bumetanide (bendroflumethiazide 10 mg theophylline ethylenediamine 400 mg) (6 pats)	K	Na
	K	Na
	Cl	Na
	Cl	Na
	H_2O	Na
	H_2O	Na
	H_2O	Cl
	H_2O	Cl
	Na+K	Cl
	Na+K	Cl

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Absorption and Elimination of D-Propoxyphene, Acetyl Salicylic Acid, and Phenazone in a Combination Tablet (Doleron®) Comparison Between Young and Elderly Subjects

A Melander N O Bodin K Danielson B Gustafsson G Haglund and D Westerlund

From the Department of Clinical Pharmacology University of Lund Lund the Unit for Community Care Sciences Dalby the Medical Department and the Research Laboratories Astra Lakemedel and Astra Pharmaceuticals Sodertalje Sweden

ABSTRACT The single-dose kinetics of D-propoxyphene, acetyl salicylic acid and phenazone, given in a combination tablet (Doleron®), were compared in young and elderly subjects Serial blood samples were taken 0–48 hours after administration The plasma concentrations of propoxyphene and of its major metabolite, norpropoxyphene, were assessed by mass fragmentography, those of phenazone by gas chromatography, and those of acetyl salicylic acid plus salicylic acid by spectrofluorometry Neither for propoxyphene, norpropoxyphene, acetyl salicylic acid nor phenazone did the areas under the concentration curves or the elimination half lives differ between young and elderly subjects These data do not provide pharmacokinetic support for a general reduction of the Doleron dosage in elderly subjects

The major drug consumers are found among elderly subjects with respect to both frequency of prescriptions and number of drugs prescribed It is often noted that elderly subjects are more susceptible to drugs than are young individuals This could be due to an increased tissue sensitivity to drugs in the elderly but it may also result from a reduced capacity for drug elimination with increasing age Indeed recent studies suggest such a reduction for a few drugs (1 4 5)

However information on the kinetics of drugs in elderly subjects is still sparse Therefore an attempt has been made to compare single-dose kinetics of various common drugs in young and elderly subjects The present study concerns Doleron® (Astra Lakemedel AB Sodertalje Sweden) which contains D-propoxyphene chloride 65 mg acetyl salicylic acid 350 mg phenazone 150 mg caffeine 50 mg and Transergan® 5 mg In addition the blood concentrations of norpropoxyphene the demethylated major metabolite propoxyphene were measured

SUBJECTS AND METHODS

Subjects

The study comprised one group of eight young and one group of six elderly volunteers The data derived from the young subjects have been presented in a preceding report concerning the influence of food intake on the bioavailability of Doleron® (3) All subjects studied were drug-free and healthy as judged clinically and by numerous laboratory tests The elderly subjects were recruited from a population of pensioners voluntarily subjected to an extensive yearly clinical and laboratory examination at the Unit for Community Care Sciences in Dalby Sweden They (four males and two females) were 74 and 75 years old and weighed 65–88 kg The age and weight ranges of the young subjects (five males and three females) were 24–37 years and 56–85 kg respectively Each subject was extensively informed and gave written consent The investigation was approved by the Ethical Committee of the University of Lund

Drug intake and blood sampling

One Doleron tablet was ingested at about 8 a m together with a standardized breakfast meal as recently described (3) Blood was sampled via an indwelling antebrachial polyethene cannula, the samples (about 10 ml) being obtained before (0 hour) and at 15 30 45 60 75 90 120 240 360 480 min 24 and 48 hours after drug ingestion The exact time of blood sampling (when the sampling tube was half filled) was recorded and used in calculations and graphs Plasma was collected and frozen in three different portions at −20°C until assessed for its content of pro-

Table I *Kinetic data for plasma propoxyphene in elderly and young healthy subjects following intake of one Doleron® tablet together with a standardized breakfast meal*

Subj no	C_{max} (ng×ml⁻¹)	t_{max} (min)	AUC (ng×min ×ml⁻¹)
Elderly			
1	74	242	64 5 10³
2	32	81	19 7
3	36	44	10 6
4	88	85	38 9
5	68	118	29 0[b]
6	137	47	26 3
Mean	73	103	31 5 10³
S D	38	73	18 7
Young[a]			
Mean	71	102	26 8 10³
S D	37	60	22 0
Statistical significance of differences between elderly and young subjects	N S	N S	N S

[a] Melander et al (3) [b] Measured during 24 h

Table II *Kinetic data for plasma norpropoxyphene in elderly and young healthy subjects following intake of one Doleron® tablet together with a standardized breakfast meal*

Subj no	C_{max} (ng×ml⁻¹)	t_{max} (min)	AUC (ng×min ×ml⁻¹)
Elderly			
1	102	58	107 1 10³
2	57	424	98 2
3	58	97	60 9
4	62	85	83 1
5	71	118	97 2
6	103	76	114 5
Mean	76	143	93 5 10³
S D	21	139	19 2
Young			
Mean	64	187	77 4 10³
S D	21	102	25 9
Statistical significance of differences between elderly and young subjects	N S	N S	N S

[a] Melander et al (3)

poxyphene norpropoxyphene acetyl salicylic acid plus salicylic acid and phenazone

Analytic methods and calculations

The plasma concentrations of propoxyphene norpropoxyphene acetyl salicylic acid plus salicylic acid and phenazone were measured by mass fragmentography fluorometry and gas chromatography as described in the preceding paper (3) The concentrations of the respective compounds were plotted against time and the peak concentrations (C_{max}) and elimination half lives were estimated The area under the plasma concentration curve (AUC) was calculated by the method of overlapping parabolas (2) including the infinite area The statistical significance of differences was calculated by Student s *t* test

RESULTS

Propoxyphene and norpropoxyphene

In the elderly subjects (Table I) the peak concentrations of propoxyphene in plasma showed a pronounced interindividual variation ranging from 32 to 137 ng/ml The time to reach peak concentrations varied between 1 and 4 hours Also the AUC values displayed a considerable variation between individuals being about 6-fold

These results were very similar to those previously recorded in the young subjects (C_{max} range 29–130 ng/ml, t_{max} 1–4 hours AUC variation about 7 fold) Neither the respective mean AUC values differed significantly (Table I) As the distribution of propoxyphene seemed to continue throughout the sampling period and no apparent elimination equilibrium was achieved no effort was made to assess elimination half life values

Like those of propoxyphene the parameters of norpropoxyphene were similar in young and elderly subjects (Table II)

Acetyl salicylic and salicylic acid

The respective parameters for acetyl salicylic acid plus salicylic acid were similar in young and elderly subjects and showed only a small interindividual variation (Table III) Thus the mean peak concentrations were 21 μg/ml in both groups the mean times to reach these peak concentrations were 140 min (young) and 142 min (elderly) and the respective mean AUC values were 6 8 (young) and 7 8 (elderly)×10³ μg×min×ml⁻¹

Phenazone

In the elderly subjects the peak concentrations of phenazone varied about two-fold (range 3 1–6 4 μg/ml) while the time to reach peak concentrations ranged from 20 to 125 min (Table IV) The elimination half lives ranged from 12 5 to 25 9 hours and

Table III *Kinetic data for plasma acetyl salicylic acid and salicylic acid in elderly and young healthy subjects following intake of one Doleron® tablet together with a standardized breakfast meal*

Subj no	C_{max} ($\mu g \times ml^{-1}$)	t_m (min)	AUC ($\mu g \times min \times ml^{-1}$)
Elderly			
1	23 9	242	7 9 10³
2	21 1	128	8 5
3	18 5	123	7 7
4	25 2	124	9 4
5	19 1	118	7 1
6	15 4	117	6 3
Mean	21	142	7 8 10³
S D	4	49	1 1
Young[a]			
Mean	21	140	6 8 10³
S D	7	87	0 7
Statistical significance of differences between elderly and young subjects	N S	N S	N S

[a] Melander et al (3)

Table IV *Kinetic data for plasma phenazone in elderly and young healthy subjects following intake of one Doleron® tablet together with a standardized breakfast meal*

Subj no	C_{max} ($\mu g \times m^{-1}$)	t_{max} (min)	AUC ($\mu g \times min \times ml^{-1}$)	$t_{\frac{1}{2}}$ (h)
Elderly				
1	3 9	125	9 3 10³	25 9
2	4 2	45	7 2	22 6
3	3 5	45	4 1	22 5
4	3 1	45	6 4	32 5
5	4 1	80	4 0	12 5
6	6 4	20	5 9	18 1
Mean	4 2	60	6 1 10³	22 3
S D	1 2	37	2 0	6 7
Young				
Mean	3 3	70	4 5	16 8
S D	1 0	49	2 6	7 7
Statistical significance of differences between elderly and young subjects	N S	N S	N S	N S

[a] Melander et al (3)

the AUC values from 4 0 to 9 3×10³μg×min×ml⁻¹ (Table IV) Except for the time to reach peak concentration each parameter displayed a higher mean value in elderly than in young subjects (Table IV) However in neither case was the difference statistically significant

DISCUSSION

The findings in the present study seem to indicate that there is no major systematic difference between young and elderly subjects in the kinetics of propoxyphene acetyl salicylic acid or phenazone when these compounds are administered as a single tablet of the combination preparation Doleron® This does not provide a kinetic basis for a general reduction of the Doleron dosage in elderly subjects

The present findings contrast in part with studies on other preparations age differences in the disposition of phenazone have been recorded (1) In a study with geriatric patients the plasma half life values obtained with phenazone were 45% longer compared with younger volunteers (4) No previous information seems to be available as to possible age differences with respect to acetyl salicylic acid propoxyphene or its major metabolite norpropoxyphene

Not only the young subjects but also elderly individuals in the present study were healthy and drug free Indeed the elderly subjects were selected from a population of pensioners undergoing a very extensive yearly health control as part of an investigation at the Community Care Center in Dalby Sweden Such conditions have not been the rule in other studies concerning possible age differences in drug disposal and this may help to explain the discrepancy between the present and previous data

It should be admitted on the other hand that the current material is small and hence would only disclose rather large differences Indeed the numerical mean AUC values for each compound were greater in the elderly than in the young subjects although the differences did not reach statistical significance In addition a numerically—but not significantly—longer mean plasma half life for phenazone was recorded in the elderly subjects However irrespective of the possible age differences the interindividual variations within both young and elderly subjects were much greater Therefore the major issue in Doleron therapy is the individualization of dosage (3)

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Platelet Aggregation in Diabetes Mellitus

Hans Draminsky Petersen and Johs Gormsen

From Department T Bispebjerg Hospital and the Coagulation Laboratory Municipal Hospital Copenhagen Denmark

ABSTRACT Thirty-eight patients with juvenile diabetes mellitus, aged 21–77, were tested for platelet aggregation in vitro Vascular complications were found in 20 patients and diabetic retinopathy in 16 of these All patients received their usual dose of insulin in the morning on the day of the examinations which were carried out shortly before or 2–3 hours after lunch Ninety normal controls were tested at the same time of day The aggregation was estimated turbidometrically and defined by the threshold concentration of adenosine diphosphate or adrenaline that produced a secondary aggregation with a light transmission not less than 80% of that given by the platelet poor plasma No significant differences could be demonstrated in platelet aggregation between the normal controls and the patients with diabetes mellitus or any subgroup of these No correlation was found between the threshold concentrations and the plasma levels of glucose or β hydroxybutyric acid

Many studies have been performed in recent years to illustrate the significance of platelets in the development of arterial thromboatherosclerosis Shortened platelet survival time and/or some kind of platelet hyperactivity have been found in patients with transient cerebral ischemia (TCI) ischemic heart disease (IHD) or peripheral thromboatherosclerosis (PTA) (22 31)

If platelets are of any significance for the above disorders they might also be involved in the development of diabetic macro- and microangiopathy (21) The conclusions from studies in diabetic patients are contradictory Some authors report normal aggregation (13 16) some find hyperactive platelets (7 19 27) and some believe that any increased platelet activity is due to a plasma factor (18)

The aim of the present work has been to study the platelet aggregation in vitro in patients with diabetes using a technique that has proved valuable in other studies in which we have demonstrated a distinct increase in adenosine diphosphate (ADP)-induced aggregation in patients suffering from TCI (2) IHD and PTA (11) and in patients recovering after acute cerebral infarction (20) The patients have been examined after taking their insulin and meals as usual The normal controls were tested at the same time of day likewise without fasting

PLATELET AGGREGATION STUDIES

Platelet aggregation was evaluated turbidometrically according to the principles described by Born (4) The experiments were performed in a Payton aggregometer model 300 (stirring at 900 r p m) at 37°C Blood was drawn by puncture of a cubital vein after slight compression 9 parts of blood being kept in a plastic tube with 1 part of trisodium citrate 3 13% Platelet rich and platelet poor plasma was obtained by immediate centrifugation at room temperature at $200\times g$ for 3 min and $1000\times g$ for 20 min respectively The plasma was pipetted by plastic material into other plastic tubes kept capped at room temperature for not more than one hour and prewarmed at 37°C for 3 min before testing The platelet rich plasma contained 200–350 000 platelets/µl counted in a Thomas counting chamber Platelet aggregation was started by adding ADP (Sigma Chemical Co St Louis USA) in 0 05 ml of sterile saline to 0 5 ml platelet rich plasma The final concentrations of ADP were 0 25 0 50 1 0 2 0 and 5 0 µM Platelet aggregation was also started by adding adrenaline (Gauche Rhône Poulenc Paris) giving final concentrations of 0 01 0 05 0 5 and 1 0 µg/ml Estimates were made of the threshold concentration which is the lowest concentration of ADP or adrenaline that is able to produce a secondary aggregation with an amplitude corresponding to not less than 80% of that obtained with the transmission given by the platelet poor plasma (11)

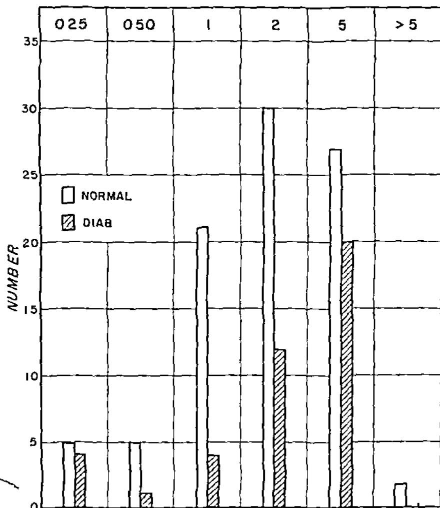


Fig 1 Threshold concentration of ADP (μM) in normal *controls and in patients with* diabetes mellitus

Other methods

The concentrations in plasma of Hb glucose creatinine and β-hydroxybutyric acid were estimated by the Department of Clinical Chemistry Bispebjerg Hospital which also performed other routine tests

STUDY POPULATION

All individuals tested were carefully questioned about any intake of drugs which might influence platelet aggregation and were excluded if they had taken such drugs during the past week

The control material comprises 90 persons 20–70 years old all donors at the Blood Bank of the Municipal Hospital of Copenhagen Most samples were taken between 10 a.m. and 2 p m

The patient group comprises 38 patients (21 men and 17 women) 21–77 years old with juvenile diabetes mellitus All patients were treated with insulin most with Insulin Retard® (Leo) which was given as usual in the morning on the day of the examination. Most of the patients were examined between 1 and 4 p m a few before noon and they were not fasting

Diagnosis Diabetic retinopathy was diagnosed on the basis of ophthalmologic criteria defined by one of the authors (H P D) The diagnosis of nephropathy was based on persisting proteinuria or elevated serum creatinine for more than one year and the diagnoses of atherosclerosis and diabetic neuropathy were based on clinical examination and ECG Eighteen of the 38 patients were without any vascular complications according to our definition Of the 20 patients with vascular complications diabetic retinopathy was found in 16 of whom 5 had nephropathy and 10 neuropathy and distinct arterial insufficiency in 6

RESULTS

Platelet aggregation studies

The threshold concentrations of ADP and adrenaline in the controls and the diabetic patients are given in Figs 1 and 2 The threshold concentration of ADP was ≤1 μM in 33 % of the controls and in 21 %

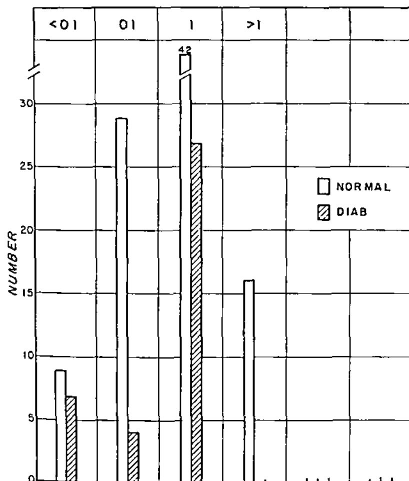


Fig 2 Threshold concentration of adrenaline in plasma (μg/ml) in normal controls and in patients with diabetes mellitus

of the patients The threshold concentration of adrenaline was ≤0 1 μg/ml in 40% of the controls and 27% of the patients Accordingly no hyperaggregability was found in the patients with this technique

No significant differences were detected in the threshold concentrations of either ADP or adrenaline between the control group and the diabetic group (Figs 3 and 4) nor between the control group and any subgroup of patients with diabetic complications

No correlation was found between the threshold concentration and blood levels of glucose or β-hydroxybutyric acid

The correlation between threshold concentrations of ADP and adrenaline was identical in the controls and the diabetics The differences between light transmission in platelet rich and platelet poor plasma were identical in both groups and the results were not due to hyperlipidemia

DISCUSSION

Abnormalities in the hemostatic balance might be of some significance for the development of diabetic macro- and microangiopathy (21) Almer and Pandolfi (1) found a reduced fibrinolytic activity and Pandolfi et al (24) an increased von Willebrand factor activity in patients with diabetic retinopathy Platelet thrombi were demonstrated by Fagerberg (9) in the small vessels of sural nerve biopsy in diabetic neuropathy by Timperley et al (28) in the cerebral microcirculation in patients dying from ketoacidosis and by Bloodworth and Monitor (3) in the retinal vessels in experimental canine diabetic retinopathy Ferguson et al (10) showed increased platelet and fibrinogen turnover in patients with diabetes mellitus

Studies on platelet aggregation in vivo and in vitro in diabetic patients have been carried out for years They have been performed in the morning on fasting patients Heath et al (16) found an increased

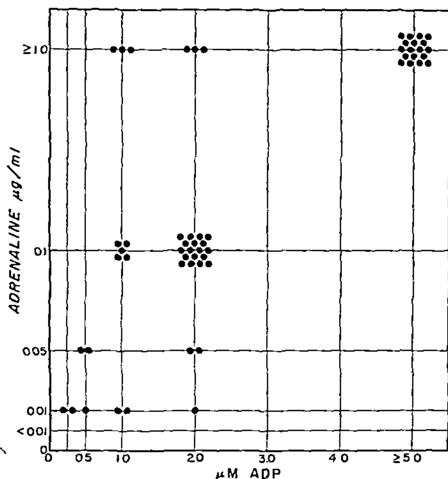


Fig 3 Correlation between ADP and adrenaline threshold concentrations in normal controls

aggregation tendency in vitro defined by a broader amplitude after adding ADP in low concentrations (1–2 µM) and by a decrease in disaggregation in patients with severe diabetic retinopathy Hassanein et al (15) found decreased disaggregation after addition of ADP in diabetics unrelated to vascular complications An i v injection of insulin was followed by an increase in aggregation rates The maximal amplitude however was reduced and the disaggregation increased The decrease in the primary disaggregation was especially marked in patients with high cholesterol levels Kwaan et al (18) showed that ADP induced (but not adrenaline induced) aggregation of normal platelet rich plasma was increased by adding plasma from patients with diabetes especially if the diabetics had vascular complications They found no relationship between the increase in aggregation tendency and age or weight dose of insulin and levels of blood glucose triglyceride cholesterol free fatty acids or albumin They also noted that aggregation was not affected by *sulfonylurea i v glucagon or insulin*

Neither did aspirin affect the aggregation tendency Sagel et al (26) found an increased aggregation tendency determined as an increase in light transmission after 4 min using low concentrations of ADP (0 125 and 0 25 µM) or low concentrations of adrenaline and collagen in platelet rich plasma from diabetics unrelated to presence of vascular complications There was no relation between fasting blood glucose and the aggregation tendency The increase was reduced by aspirin i v tolbutamide or peroral glucose whereas sulfonylurea perorally had no effect O Malley et al (23) found lower threshold concentrations of ADP and adrenaline in 20 patients with diabetic peripheral neuropathy but no significant differences between the controls and the diabetic group without vascular complications They observed like Breddin et al (5) and Wu and Hoak (30) an increased tendency to formation of spontaneous platelet aggregates in vivo or in vitro Colwell et al (7) demonstrated an increased sensitivity to ADP and adrenaline unrelated to vascular complications but related to increased von Willebrand

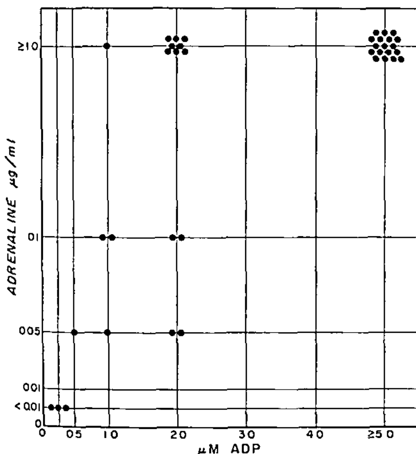


Fig 4 Correlation between ADP and adrenaline threshold concentrations in patients with diabetes mellitus

factor activity and increased blood concentrations of growth hormone They also demonstrated that diabetic platelets produced more PGE_2 like material than normal platelets (14) Passa et al (25) normalized the increased ADP aggregation in diabetic retinopathy by hypophysectomy The reports are accordingly rather conflicting Also some authors (13) have not been able to find significant changes in platelet aggregations in patients with diabetes mellitus

We have previously demonstrated a significant increase in ADP induced platelet aggregation as defined by lower threshold concentrations in patients with TCI (2) IHD and PTA (11) and after acute cerebral infarction (20) We applied the same technique in the present study in diabetic patients

All our patients suffered from juvenile diabetes mellitus 20 with vascular complications and 16 of these had diabetic retinopathy Patients were tested during the day not fasting having taken their usual dose of insulin. Normal controls were tested at the same time of day also not fasting

With this procedure we found that the aggregation tendency was not increased as defined by our criteria The threshold concentrations of ADP and adrenaline were not lower in the diabetic group whether vascular complications were present or not The amplitude will be reduced in cases of hyperlipidemia but this was not the reason for the lack of a demonstrable increase in our study

We found no correlation between aggregation tendency and plasma levels of glucose β hydroxy butyric acid factors illustrating the regulation of the metabolism Diurnal fluctuations in aggregation if present could hardly be due to fluctuations in the plasma concentrations of glucose potassium lipoproteins corticosteroids glucagon or growth hormone (6 11 12 29) We found that an i v infusion of somatostatin did not influence platelet aggregation (8)

Changes in levels of catecholamines during hyper or hypoglycemia might influence the aggregability Whether changes in the plasma level of the physiological aggregation inhibitor 2 3-diphospho-

glycerate the concentration of which is increased in diabetic plasma (17) may influence our results is also unknown to us at present

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Prognosis for Juvenile Diabetics with Nephropathy and Failing Renal Function

Allan Renard Andersen Jane Kock Andersen Jens Sandahl Christiansen and Torsten Deckert

From the Steno Memorial Hospital Gentofte Denmark

ABSTRACT A total of 157 consecutive patients with juvenile diabetes (onset before the 31st birthday), diabetic nephropathy, and impaired renal function were followed up until 1 1 1976 All the patients had been admitted to the Steno Memorial Hospital, Copenhagen, between 1934 and 1972 Independently of the patients age at onset of diabetes, it was found that persistent proteinuria appeared after an average of 19 years, and that death ensued 5–6 years thereafter Division of the patients into two groups, according to whether the diabetes had set in before or after 1940, showed no signs of an improved prognosis during the past few decades Once the serum creatinine has started to rise, the prognosis is very grave Only 50% were alive 21 months after serum creatinine levels of 2–5 mg/100 ml had been ascertained Among patients whose serum creatinine exceeded 5 mg/100 ml, 50% succumbed in 9 months It is concluded that renal transplantation, if it is to be done, should be instituted early

It is well known that patients with long standing diabetes mellitus develop generalized angiopathy and neuropathy which may lead to organic lesions in the form of retinopathy nephropathy myocardial infarction gangrene and brain damage Diabetic nephropathy is clinically fairly silent but in most young patients it will sooner or later result in renal failure (2) So far treatment with renal transplantation has not been a routine as the state of terminal uraemia in patients with diabetic nephropathy is frequently associated with other complications of advanced diabetes such as myocardial infarction stroke gangrene neuropathy and blindness According to some studies however development of the advanced diabetic organic lesions seems to be accelerated by the uraemia (4) This raises the question of whether the development of these disabling lesions can be delayed by performing renal transplantation on juvenile diabetics exhibiting nephropathy at an early stage of the uraemia However there have been only a few reports on the prognosis of diabetic nephropathy in juvenile diabetics once failing renal function has been diagnosed by methods of clinical chemistry (5 7)

As knowledge on this item is needed to assess whether it is reasonable to consider renal transplantation in the treatment of diabetic nephropathy and if so at which state of the progress of the disease it should be applied we analysed the course of the disease in 157 patients with diabetic nephropathy in whom renal failure had been verified

Reprint requests to T Deckert M D Niels Steensens Hospital DK 2820 Gentofte Denmark

STUDY POPULATION AND METHOD

The study comprises all patients with juvenile diabetes mellitus (onset before the 31st birthday) who had diabetic nephropathy with impaired renal function when admitted to the Steno Memorial Hospital between 1934 and 1972 Of the 157 patients 100 (64%) were males and 57 (36%) females male female ratio 1 75 1 In 56 patients diabetes mellitus had been diagnosed between the ages of 0 and 10 years in 61 between 11 and 20 and in 40 between 21 and 30 years of age All were on insulin

Diabetic nephropathy was defined as persistent proteinuria (proteinuria at four successive controls with an interval of at least one month) in a diabetic without clinical evidence or a history of other renal disease The diagnosis of proteinuria was confirmed by qualitative examination of the 24-hour urine using Heller's test (1) (after 1965 Albustix) and measured quantitatively by the Tsuchiya

Table I *Intervals between onset of diabetes and proteinuria and between start of proteinuria and death*

	Age (y) at onset of diabetes			
	0–10	11–20	21–30	0–30
No of years between onset of diabetes and persistent proteinuria				
Mean±S D	19 1±8 8	18 2±5 7	19 7±7 9	18 9±6 9
Range	(0) 5–37*	7–33	4–41	(0) 4–41
No of years between start of persistent proteinuria and death				
Mean±S D	6 8±6 6	5 2±4 9	4 4±5 8	5 6±5 5
Range	0–32	0–20	0–19	0–32

* One patient had proteinuria on first admission (at onset of disease) No information available until next admission in terminal uraemia.

Table II *Causes of death in 157 patients with juvenile diabetes diabetic nephropathy and increased serum creatinine*

	n	%
Uraemia	119	76
Acute myocardial infarction	14	9
Pulmonary oedema	6	4
Pneumonia	5	3
Pericarditis	3	2
Stroke	3	2
Others	7	4

(1) Impaired renal function was diagnosed when patients persistently had serum creatinine levels of 0 mg/100 ml or serum urea levels of ≥60 mg/100 ml These values were chosen in order to disregard minor variations in kidney function Serum creatinine was determined by Jaffe s reaction after protein precipitation and serum urea by the van Slyke method (1)

Fig 1 gives the distribution of serum creatinine and serum urea levels at first admission Initial serum creatinine levels of 2 0–4 0 mg/100 ml were found in 123 (78%) of the patients (serum urea 60–149 mg/100 ml)

The analysis was concluded on 1 1 1976 At that time all the patients had died Sex age time of onset of diabetes mellitus start of persistent proteinuria and time of death were recorded So were the serum urea and serum creatinine values the cause of death given on the death certificate and whether blindness had been diagnosed by an ophthalmologist

RESULTS

The number of years elapsing from the onset of diabetes until the finding of persisting proteinuria and from the diagnosis of proteinuria until death are given in Table I Despite considerable individual variation it is seen that proteinuria sets in and death occurs after the lapse of about 18–20 years and about 23–26 years respectively independently

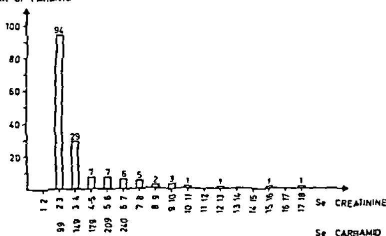


Fig 1 Renal function expressed in serum creatinine or serum urea at the beginning of the study of 157 juvenile diabetics with diabetic nephropathy and impaired renal function

Table III *Prognosis in diabetic nephropathy with impaired renal function before and during the penicillin era*

Diabetes mellitus diagnosed	Before 1941	After 1940
No of years between onset of diabetes and persistent proteinuria		
Mean±S D	21 0±7 9	14 3±5 5
Range	(0) 5–37*	4–25
No of years between start of persistent proteinuria and death		
Mean±S D	5 7±5 7	5 3+4 9
Range	0–32	0–16
No of years between diagnosis of impaired renal function and death[b]		
Mean±S D	1 4±1 8	1 8+1 6
Range	0–12	0–9

One patient had proteinuria on first admission (at onset of disease) No information available until next admission in terminal uraemia

[b] Serum creatinine ≥2 0 mg/100 ml serum urea ≥60 0 mg/100 ml

of age at the time of diagnosis The prognosis proved to be the same for both sexes

All the patients had died before the end of 1975 The causes of death are presented in Table II Uraemia was stated as the cause of 76% of the deaths Other causes of death were particularly pulmonary oedema stroke pneumonia and pericarditis

Forty-one (41%) of the males and 25 (44%) of the females i e 66 (42%) of the 157 patients became blind

Fig 2 gives the mortality rate for the total series It shows that 50% had died 18 months 83% three years and 95% six years after the examination which first disclosed serum creatinine levels of ≥2 mg/100 ml or serum urea levels of ≥60 mg/100 ml Fig 3 sets out the mortality rate for patients whose serum creatinine level was 2–5 mg/100 ml and for patients in whom it exceeded 5 0 mg/100 ml Of the patients with serum creatinine levels between 2 and 5 mg/100 ml 50% were alive 21 months 18% three years and 5% 11 years after the examination which first revealed serum creatinine ≥2 0 mg/100 ml or serum urea ≥60 mg/100 ml Of the patients with serum creatinine levels above 5 mg/100 ml 50% had died in 9 months and all had died in 3 years

Division of the series according to onset of diabetes before and after 1940 showed that patients whose diabetes had been diagnosed prior to 1941 had a longer duration of diabetes before the occurrence of persistent proteinuria than patients with an onset of diabetes after 1940 (Table III)

DISCUSSION

The ratio males females (1 75 1) in the present series is surprising as the incidence of juvenile diabetes mellitus is only about 20% higher in males than in females Whether diabetic males are more apt than diabetic females to develop nephropathy is not known

Age at onset of diabetes proved to have no influence on the number of years elapsing until proteinuria occurred or until death That juvenile diabetics developing renal complications acquire persistent proteinuria after having had diabetes for about 18–20 years and that death occurs 5–6 years thereafter accords with the course described by Kussman et al (6) Knowles (5) and Shapiro et al (7) but the prognosis is not so poor as found by Wilson et al (9)

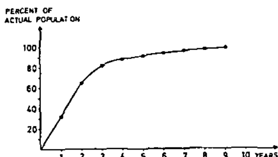


Fig 2 Cumulative death rate for 157 juvenile diabetics with diabetic nephropathy after impaired renal function (serum creatinine ≥2.0 mg/100 ml or serum urea ≥60 mg/100 ml) had been diagnosed

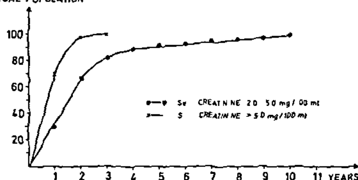


Fig 3 Cumulative death rate for juvenile diabetics with diabetic nephropathy after impaired renal function had been diagnosed n=110 (●) n=55 (×)

Evaluation of the dependence of the prognosis upon whether the diabetes set in before or after 1940 (before and during the antibiotic era) showed that patients who have developed their diabetes *after 1940 apparently* are *worse off than those with* an onset prior to 1940 the latter dying after an average duration of 26 3 years and the former after 19 6 years (Table III) These values presumably indicate that admission of patients with severe nephropathy to the Steno Memorial Hospital was not as common before as after 1940

That the survival time after the appearance of persistent proteinuria and incipient impairment of renal function was unchanged in patients whose diabetes set in before or after 1940 (Table III) shows that the more intensive conservative treatment of the uraemia during the past few decades has not given better results

Acute myocardial infarction proved to be the cause of death in only 9% of the patients This rate *is considerably below the percentage of 38% in* the series of Kussman et al (6) But their series was smaller and the follow up period appreciably shorter

That 42% of the patients became blind confirms previous findings (4) showing that only 2 years passed before 50% of juvenile diabetics with proliferative retinopathy and proteinuria became blind as compared with 5 years for 50% of those without proteinuria Only 12% of juvenile diabetics with normal serum creatinine became blind after a duration of diabetes of more than 40 years (3)

The present study has confirmed that diabetic nephropathy is a very serious disease and that the prognosis is exceptionally poor when renal function starts failing Intensive nephrological treatment of this group of patients is still in an experimental stage and the indication for renal transplantation *in diabetic nephropathy is a matter of discussion* However the present material indicates that intensive treatment including transplantation should be instituted earlier in diabetic nephropathy than in renal failure due to other causes

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Overweight in Women—Metabolic Aspects

The Population Study of Women in Goteborg 1968–1969

Henry Noppa Calle Bengtsson Per Bjorntorp
Ulf Smith and Elisabeth Tibblin

From the Departments of Medicine and Clinical Chemistry Sahlgren s Hospital University of Goteborg Goteborg Sweden

ABSTRACT In a population sample of 1462 women aged 38–60 years, those with overweight were studied separately and compared with the women in the total population sample Overweight was defined as the upper 5% of a weight index in the various ages studied and the weight index as

$$\frac{\text{body weight (kg)} \quad 100}{\text{body height (cm)} - 100}$$

Significant differences, with higher values in the overweight women, were found for serum triglycerides, serum uric acid and arterial BP Smoking was significantly less common in the overweight women Serum cholesterol was similar in overweight women and in women in the total sample Higher values for some risk factors for ischaemic heart disease in the overweight group of women thus seemed to be compensated to some extent by a lower number of smokers in this group

Early reports from the Framingham study indicated that angina pectoris and sudden death were overrepresented in overweight subjects while non fatal myocardial infarction (MI) was not (15) In the Framingham autopsy series body weight was more strikingly related to left ventricular weight and thickness than to coronary atherosclerosis and the data were considered to indicate that obesity might represent more a haemodynamic than an atherogenic threat to the circulatory apparatus (14) However the Framingham study (14 15) also showed that coronary mortality in general and sudden death rates in particular were substantially increased in the obese Data on cardiovascular morbidity in men revealed that all types of coronary attacks were related to obesity though less strikingly in the case of MI Similarly in women angina pectoris was significantly related to body weight while MI was not

Various so-called risk factors for ischaemic heart disease (IHD) are overrepresented in overweight people (14) such as arterial hypertension (10) diabetes (8) and hypertriglyceridaemia (4 9)

Multivariate analyses of the Framingham data including possible atherogenic traits such as BP serum cholesterol blood glucose and smoking indicated that very little of the effect of obesity in promoting IHD in men could be attributed to coexisting atherogenic traits while a great deal of the effect in women with angina pectoris appeared to be mediated through such associated atherogenic traits (14)

Several studies have dealt with metabolic disturbances in overweight or obese subjects However such series of obese people usually comprise subjects seeking medical advice because of overweight and are thus not representative for obese people in the general population Most overweight people probably do not seek medical advice for this condition Consequently little is known about the ordinary overweight subject in the general population

In 1968–69 we studied a population sample of women in Goteborg who were representative for the general population of women in Goteborg in the ages studied (2) This means that the overweight women participating in the population study were likewise representative for the overweight women in the general population Thus it is possible to compare overweight women in the general popula

Table I *Numbers of participants and ranges of weight index in overweight women (defined as women within the upper five centiles in each age stratum) and in women in the total population sample*

Age (y)	Overweight women n	Overweight women Range of weight index	Total population sample of women n	Total population sample of women Range of weight index
38	18	127–210	372	66–210
46	22	129–178	431	57–178
50	20	136–169	398	69–169
54	9	140–167	180	65–167
60	4	134–138	81	70–138
Total	73	127–210	1 462	57–210

tion with the total number of women in the general population Some preliminary results have been re ported (3) and in the present paper these studies are elucidated in more detail

STUDY POPULATION

A population study of women was carried out in Goteborg Sweden in 1968–69 (2) Altogether 1 462 wom en in five age strata were studied (Table I) with a icipation rate of 90 1% Women born on dates which e pre selected multiples of six (6 12 etc) were called the study The sample was obtained from the Revenue ffice Register Those born at the beginning of the year were called first The survey was performed for the most part during a 12 month period In this way the influence of age differences within each age group was reduced as far as possible

METHODS

The women were asked to attend the examination after an overnight fast but were allowed water in the morning The research staff was the same during the whole examination period

As examples of attempts made to avoid interobserver variations one and the same doctor measured all the BPs another made all the anthropometric measurements Body height was measured to the nearest 0 5 cm with the subject in the standing position without shoes and the feet together with the heels against the wall to which the instru ment for measuring height was fixed The women wore only knickers when they were weighed Body weight was measured to the nearest 0 1 kg Knee and wrist epicondy lar widths were measured by means of a spreading caliper and recorded to the nearest mm

History concerning angina pectoris was recorded ac cording to Rose (20) History of diabetes gallstone and renal stone disease and family history of diabetes were also recorded Diabetes was considered to be present if the subject received treatment for diabetes or had laboratory signs of diabetes (fasting blood glucose concentration exceeding 6 7 mmol/l and glucosuria) at the time of examination The participants were asked about smoking and alcohol consumption habits

BPs were measured by means of a mercury manometer with the woman sitting comfortably in a chair after about 5 min rest A 30×12 cm cuff with a nylon hooklet binding was applied firmly and evenly to the right arm The BPs were read to the nearest 2 mmHg (1) Presence of xanthelasmata was recorded

Serum cholesterol and serum triglycerides were de termined according to Levine and Zak (17) and Loftlane (18) respectively and serum uric acid by means of an enzymatic method mainly according to Praetorius (19)

Blood glucose was measured by means of a glucose oxidase method according to Levin and Linde (16)

Weight index was defined as

$$\frac{\text{body weight (kg)} \quad 100}{\text{body height (cm)} - 100}$$

The distributions of the weight indices in the various age strata are shown in Fig 1

Overweight was defined as a weight index within the upper five centiles of the weight indices in the various age strata With this definition 73 women were classified as overweight thus constituting 5% of the total population sample who had attended the examination The weight index ranges of women defined as overweight as well as of women in the total population sample are shown in Table I

Statistical methods Conventional statistical methods were used for calculating mean values standard devia tions and correlation coefficients (*r*) regression coeffi

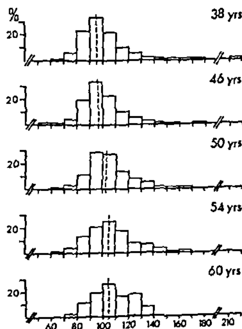


Fig 1 Distribution of weight indices

Table II *Body height femur and wrist epicondylar widths (right leg and arm) in overweight women (n=73) and women in the total population sample (n=1462)*

	Overweight women		Total population sample of women		Statistical significance
	Mean	S D	Mean	S D	
Body height (cm)	162 9	6 2	163 6	6 3	N S
Epicondylar width (cm)					
Femur	9 5	0 6	8 9	0 5	$p<0.01$
Wrist	5 3	0 3	5 1	0 3	N S ($p<0.10$)

N S = Not significant

cients and significance of regression coefficients The hypothesis of differences in frequencies between groups was tested by means of the χ^2 test comparing those who were classified as overweight with those who were not Significance of differences between mean values was tested with Student s *t* test (two-tailed test) When testing mean values comparisons were made between women classified as overweight and women in the total population sample including those who were classified as over weight In this way differences between the overweight individuals and the others may have been slightly under estimated The differences were considered statistically significant at $p<0.05$

RESULTS

Anthropometric data other than weight index in overweight women

Some other anthropometric data of women with a weight index within the upper five centiles and thus defined as overweight are presented in Table II Mean body height was similar to that of the total population sample while femur epicondylar width was significantly larger and wrist epicondylar width tended to be larger in overweight women

Prevalence of angina pectoris in overweight women

As MI is rare in young and middle aged women it is not possible to compare the prevalence of myocardial infarction in the two groups However angina pectoris is more common in women and 29 women in the total population sample reported a history of angina pectoris Angina pectoris was recorded in 2 (3%) of 73 overweight women and in 27 (2%) of 1389 non obese women The difference was not statistically significant

Possible risk factors for ischaemic heart disease

Tables III and IV present data concerning the prevalence of some possible risk factors for IHD in overweight women and in women in the total population sample

Diabetes mellitus Two (3%) of the overweight women had diabetes mellitus compared with 12 (1%) of the total population sample The difference was not statistically significant (Table III) Overweight women tended to have higher blood glucose values ($p<0.10$ Table IV) than women in the total population sample A family history of diabetes among the parents was reported in 16% of overweight women and in 11% of women in the total population sample (difference not significant)

Hyperlipidaemia Similar values were found for serum cholesterol in overweight women and in women in the general population while serum triglycerides were markedly higher in the overweight women ($p<0.001$ Table IV)

Arterial hypertension Overweight women took antihypertensive drugs more often than women in the general population ($p<0.001$ Table III) They also had higher mean BPs ($p<0.001$ Table IV)

Table III *Data on diabetes arterial hypertension smoking and alcohol consumption in overweight women (n=73) compared with women in the total population sample (n=1462)*

	Overweight women (%)	Total population sample of women (%)	Statistical significance
Diabetes mellitus	3	1	N S
Antihypertensive treatment	16	5	$p<0.001$
Smoking	25	41	$p<0.01$
Beer ≥once a week	30	49	$p<0.01$
Wine ≥once a week	10	18	$p<0.05$
Spirits ≥once a week	3	6	N S

N S = Not significant

Table IV *Fasting blood glucose serum lipids BP and serum uric acid in overweight women in relation to the total population sample*

Age (y)	Overweight women			Total population sample of women			Statistical significance
	n	Mean	S D	*n*	Mean	S D	
Fasting blood glucose (mmol/l)							
38	17	4 2	0 6	371	4 0	0 7	N S
46	22	4 3	0 8	431	4 1	0 7	N S
50	20	4 8	1 6	397	4 2	1 0	$p<0\ 01$
54	9	4 8	0 9	179	4 3	1 0	N S
60	4	4 8	1 5	81	4 3	1 7	N S
Total	72	4 5	1 1	1 459	4 1	0 9	N S ($p<0\ 10$)
Serum cholesterol (mmol/l)							
38	18	6 5	1 0	371	6 3	0 9	N S
46	22	7 3	1 2	429	6 8	1 4	N S ($p<0\ 10$)
50	20	7 5	1 0	398	7 2	1 1	N S
54	9	6 7	1 0	178	7 4	1 1	N S ($p<0\ 10$)
60	4	7 8	0 2	81	7 4	0 9	N S
Total	73	7 1	1 1	1 457	6 9	1 2	N S
Serum triglycerides (mmol/l)							
38	18	1 4	0 4	371	1 1	0 4	$p<0\ 05$
46	22	1 4	0 6	430	1 2	0 7	N S
50	20	1 5	0 8	398	1 3	0 6	N S
54	9	1 9	1 2	178	1 4	0 7	$p<0\ 05$
60	4	2 1	1 7	81	1 3	0 6	$p<0\ 05$
Total	73	1 5	0 8	1 458	1 2	0 6	$p<0\ 001$
Systolic BP in the seated position (mmHg)							
38	18	127	10	372	123	11	N S
	22	149	32	431	130	19	$p<0\ 001$
	20	157	28	397	137	22	$p<0\ 001$
	9	168	28	180	143	24	$p<0\ 01$
	4	148	10	81	154	27	N S
Total 73		148	28	1 461	133	22	$p<0\ 001$
Diastolic BP (phase 4) in the seated position (mmHg)							
38	18	88	8	372	81	9	$p<0\ 01$
46	22	93	15	431	85	11	$p<0\ 01$
50	20	98	9	397	88	11	$p<0\ 001$
54	9	104	17	180	89	12	$p<0\ 001$
60	4	90	5	81	92	12	N S
Total	73	95	13	1 461	86	11	$p<0\ 001$
Serum uric acid (µmol/l)							
38	18	321	166	368	215	72	$p<0\ 001$
46	22	265	60	429	227	66	$p<0\ 01$
50	20	249	61	396	245	72	N S
54	9	380	131	176	257	84	$p<0\ 001$
60	4	338	54	81	263	90	N S ($p<0\ 10$)
Total	73	293	111	1 450	233	78	$p<0\ 001$

N S = Not significant

Smoking Smoking was less common among the overweight women (Table III) The difference was statistically significant ($p<0\ 01$)

Alcohol consumption Overweight women consumed beer and wine less often than women in the general population (Table III) The difference was statistically significant No conclusions could be drawn concerning spirits due to the low consumption among the women studied

The data presented for the total number of wom-

Table V *Correlations between weight index and some other variables in a population sample of women (n 1462)*

	Regression equation	Correlation coefficient
Serum cholesterol	$y = 0.007x+6.162$	0.11
Serum triglycerides	$y = 0.006x+0.528$	0.21
Fasting blood glucose	$y = 0.007x+3.360$	0.16
Serum uric acid	$y = 1.080x+1$[illegible]0	0.[illegible]6
Systolic BP	$y = 0.32x+101$	0.27
Diastolic BP phase 4	$y = 0.18x+67$	0.30

All regression coefficients statistically significant ($p<0.001$)

en in the two samples in Table III agree with the results found in the separate age strata so the latter are not shown here

Hyperuricaemia Serum uric acid was significantly higher in overweight women than in women in the general population ($p<0.001$ Table IV)

There was an overrepresentation of subjects on oral diuretics among the 54 year old overweight women but not in the other age strata and not in the group of overweight women as a whole Thus the higher serum uric acid values in the overweight women could not be explained by a more common use of diuretics in this group No conclusions could be drawn concerning the prevalence of gout because of the low prevalence of gout in women

Xanthelasmata Eyelid xanthelasmata were found in 10% of overweight women and in 3% of the general population The difference was statistically significant ($p<0.01$)

Gallstone and renal stone disease A history of gallstone disease was significantly more common in overweight women than in women in the total sample (35 and 19% respectively $p<0.001$) while no significant difference was found for renal stone disease (4 and 5% respectively)

Correlations between weight index and some of the metabolic variables

The results presented above concern a group of overweight women compared with women in the total population sample Table V shows correlations between weight index and some of the variables discussed above for the total sample There is a low degree correlation between weight index and serum triglycerides (r 0.21) serum uric acid (r 0.26) arterial BP (r 0.27–0.30) serum cholesterol (r 0.11) and blood glucose (r 0.16) The regression coefficients were statistically significant

Effect of early onset of overweight

The overweight women were asked about their body weight during their schooldays Sixteen women stated that they had been more obese than their class mates while 43 considered they had been of ordinary weight when at school These two groups were compared in order to evaluate whether early adiposity influenced the metabolic variables The age distribution was similar in both groups No significant difference was found between the two groups for blood lipids serum uric acid fasting blood glucose or arterial BP (Table VI)

DISCUSSION

The present paper deals with some metabolic aspects associated with overweight in women Overweight was defined as the upper five centiles of a weight index in the various age strata This index has also been used in previous population studies of men in the same region (5)

The Framingham study showed that the relation between obesity and non fatal MI was less obvious than between obesity and other coronary heart disease and seemed to be non existent in women (14) This is in agreement with the results from a study of women with MI in Goteborg (1 3) who were of similar body height and body weight as women of the same age in the general population

Table VI *Serum lipids serum uric acid fasting blood glucose and arterial BP in overweight women—a comparison between women who were overweight already as schoolchildren (n 16) and those who were not (n 43)*

	Overweight during school		Normal during school	
	Mean	S D	Mean	S D
Cholesterol	7.1	[illegible]	7.0	1.1
Triglycerides	1.4	0.7	1.4	0.7
Uric acid	316	173	81	88
Fasting blood glucose	4.7	1.6	4.4	0.9
Systolic BP	148	9	148	[illegible]8
Diastolic BP phase 4	93	15	96	[illegible]

No statistically significant differences

An overrepresentation of angina pectoris has previously been noted in the Framingham study (14 15) but could not be demonstrated in the present series of overweight women However angina pectoris was uncommon in both obese and non-obese women The present material is therefore too small for definite conclusions concerning angina pectoris It seems reasonable that being obese and thus overloading the body might increase the haemodynamic demands e g when walking up-hill and a higher prevalence of angina pectoris in overweight subjects might thus be due to both metabolic and mechanical causes

There are several established risk factors for IHD—factors which have been shown to be statistically associated with IHD but not necessarily causative e g hyperlipidaemia arterial hypertension diabetes and smoking. If correlations are found between such factors and overweight a relationship between overweight and IHD should also be expected

Diabetes mellitus has been found to be a risk factor for MI not least in women (1) An overrepresentation of diabetics among obese subjects has been noted previously (8) but could not be demonstrated in the present study Fasting blood glucose values tended to be higher among overweight women in the present study A statistically significant difference was found in women aged 50

A relationship of a similar kind was found between MI and arterial hypertension (1) Overweight women in the present study had higher BPs than women in the general population Previous studies have indicated that higher BPs noted in overweight women are mostly genuine and not due to their larger arm circumferences (10)

Women with MI in Göteborg had higher average serum triglyceride values than women in the general population (1) Women with high serum cholesterol values were however not overrepresented in the infarction group which is in agreement with the results from Framingham (12) and Tecumseh (11) Similarly a relationship was found between overweight women and high triglycerides in the present study but not between overweight and high serum cholesterol values These results also agree with those from the Stockholm Prospective Study (9)

As factors such as diabetes arterial hypertension and hypertriglyceridaemia are more common in women who suffer a MI as well as in overweight women than in women in the general population an overrepresentation of overweight among women with MI should be expected The reason for this not being the case might at least partly be due to the fact that smokers are underrepresented among overweight women and a strong correlation has been shown between smoking and MI in women (1) There is no such correlation between smoking and angina pectoris (1 13 22) This might be one explanation why angina pectoris but not MI seems to be overrepresented among overweight subjects

Overweight women had higher serum uric acid values The reason for this elevation of serum uric acid is unclear (21) The difference could not be explained by an overrepresentation of diuretic therapy in the overweight women in this study

In contrast to what was expected for smoking habits the observation that overweight women consumed beer and wine less often than other women was somewhat surprising.

The present study thus shows a relationship between overweight and factors such as hyperglycaemia, hyperuricaemia hypertriglyceridaemia and arterial hypertension Previous studies indicate that there is a relationship between these variables and fat cell size (7) Fat cell number rather than fat cell size is increased in women who already during childhood were overweight compared with other girls of similar age (6) No differences were found when women in the present series with and without overweight during childhood were compared Fat cell measurements must however be performed in order to settle this question more precisely and this is in progress

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BOOK REVIEW

Skeletal maturity of youths 12–17 years United States National Center for Health Statistics Vital and health statistics series 11 no 160 DHEW publication no (HRA) 76–1642 90 pp Health Resources Administration DHEW, Rockville Maryland 20857 USA 1976 Single copies available free of charge from NCHS Room 8–20 5600 Fishers Lane Rockville Maryland 20857 Attn M Flaer or call (301) 443-NCHS Multiple copies for $1 55 each (prepaid) from the Superintendent of Documents US Government Printing Office Washington D C 20402 USA

This report presents national estimates of the levels of skeletal maturity of the hand wrist for non institutional ized US youths age 12–17 years based on findings from the Health Examination Survey of 1966–70 National esti mates from a corresponding study of children age 6–11 years in the US in 1963–65 have been reported earlier These national studies provide estimates of known reli ability against which future possible changes in skeletal maturation rates for the country as a whole can be judged

The skeletal maturity levels provided in this report are useful to a wide variety of health related professions Pediatricians use skeletal age to more accurately diagnose genetically determined syndromes and in the diagnosis and management of youths who are growing at unusual tes Pediatric surgeons need skeletal age assessments to imate the growth related effects of such procedures as diac surgery and to aid in selection of sites and ages for surgical induction of epiphyseal fusion in children with legs of unequal length These skeletal maturity data provide nutritionists with a sounder basis for recognizing and grading malnutrition and for assessing the effectiveness of intervention programs Human biologists need skeletal age assessments both to describe populations and to analyze the associations between skeletal maturation and skeletal elongation

In this survey a radiograph was taken of the right hand wrist of each youth The radiographs were assessed by medical students who received special training in the assessment of skeletal maturity and whose level of reliability were high when applying these techniques All the assessments were made against a single set of maturity standards for males that was prepared for the survey by Dr S I Pyle Later the values for girls were transformed for each bone separately to female equivalent values using the sex associated differences reported by Pyle et al. (1971)

As expected when all the youths are assessed against the same set of male standards the skeletal ages of the girls tend to be more advanced than those of boys The differences between the mean skeletal and chronological ages show pubertal accelerations in each sex with the maximum advancement at 15 5 years in boys and 12.5 years in girls The mean skeletal ages within chronologi cal age groups were distributed in an almost normal fashion except in older girls in whom the right ends of the distributions were truncated because the hand wrists of some of the girls had become adult

Skeletal age (hand wrist) onset of ossification epi physeal fusion, and bone specific skeletal ages by chronological age and the range of bone specific skeletal ages within individual hand wrists of boys and girls 12–17 years of age are presented in the detailed tables

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Diabetes of Excessively Long Duration with Only Minor Manifestations of Long-Term Diabetic Complications

Folke Lithner

From the Department of Medicine University of Umeå Umea Sweden

ABSTRACT A male patient with ketosis-prone diabetes of 55 years' duration is described To our knowledge, this is the longest duration of diabetes reported His daughter also has a ketosis prone diabetes The low degree of long term diabetic complications in both father and daughter is remarkable

Patients with juvenile diabetes have a decreased mean longevity (3 9 12) At 30 years duration of diabetes 90% of them show clinical signs of long term diabetic complications (13)

Patients who have had diabetes for more than 40 years have been described in detail in two studies of 92 and 73 patients respectively (6 7) None of the patients in either study most of them women had had diabetes for more than 51 years There is only a rather small number of patients with a 40-year survival the Joslin Clinic reported 17% in their patients (7) the main cause of death being vascular complications It is striking in both the reports on diabetics with a duration of illness of 40 years or more that the patients generally were in good health with only rather minor manifestations of vascular complications

In our department we have had a male patient who had had diabetes for 55 years He had no signs of retinopathy neuropathy or nephropathy To my knowledge there is no earlier report of a patient with such a long duration of diabetes It is of interest that his daughter has diabetes of 36 years duration she too is nearly free from signs of long term diabetic complications

CASE REPORTS

Case 1

Male 77 years old with diabetes of 55 years duration Neither of his parents was known to have diabetes but one brother and one sister developed diabetes of maturity onset type at 68 and 76 years of age respectively His diabetes was diagnosed in 1921 at 22 years of age He then had 8% glucose in the urine and pronounced ketonuria. For the first two years his daily treatment consisted of 125 g bread 50 g butter 100 g cream 100 g lard 700–1000 g vegetables and 100 g brandy During these two years he often had pronounced glucosuria and ketonuria

In 1923 when he was hospitalized at the Department of Medicine Serafimerlasarettet Stockholm insulin therapy was started in connection with a diabetic ketoacidotic coma He also had diabetic comas in 1928 and 1936 His daily insulin therapy of late years was 40 IU protamine zinc insulin + 20 IU regular insulin He has not kept to any dietary therapy since 1923 His diabetes was not well regulated sometimes he had pronounced glucosuria and ketonuria He had no long term diabetic complications except shin spots (5) from 1962 onwards He never had any signs of neuropathy or nephropathy The eye examinations were carried out by ophthalmologists

In 1965 he was hospitalized at our Department of Medicine due to myocardial infarction In 1976 he died at home of myocardial infarction the diagnosis being verified at postmortem examination

Case 2

Female 38 years old with diabetes of 36 years duration She is the daughter of patient 1 Her diabetes was diagnosed in 1941 at 2 years of age She then had 7 7% glucose in the urine and moderate ketonuria On several occasions during her childhood ketonuria was registered and in 1960 she was hospitalized due to diabetic ketoacidosis In 1964 she had a stillbirth During the pregnancy she had had toxemia and pronounced ketonuria. She has always been treated with protamine zinc insulin the daily dose is now 40 IU + 16 IU regular insulin She has never

kept to any dietary therapy At ambulatory controls during the last 10 years her diabetes has always been well regulated She has no signs of neuropathy or nephropathy nor has she shin spots (5) In 1966 occasional retinal microaneurysms were observed These minor background lesions have remained mainly unchanged In 1977 her eyes were examined by an ophthalmologist Only occasional microaneurysms and small hemorrhages are seen

This woman has a son 10 years old with diabetes since he was 8 years of age

DISCUSSION

So-called long term diabetic complications such as microangiopathy are generally considered to be due to the diabetic metabolic derangement (4) Siperstein (10) and Siperstein et al (11) however have asserted that patients with hyperglycemia secondary to pancreatitis do not develop microangiopathy Siperstein suggests that microangiopathy is not secondary to the diabetic metabolic derangement and that vascular disease is a primary disease in diabetics His hypothesis has been criticized (14)

Animals with experimental diabetes develop microangiopathy and it has been demonstrated that this is due to the diabetic metabolic derangement (1 2)

The occurrence of long term diabetic complications such as retinopathy and nephropathy increases with increasing duration of diabetes (12) However it is remarkable that this increase diminishes after 25 years of diabetes (7 9 12) This may be due to a shorter life of long term diabetics with complications so that diabetics with a very long duration of the disease are a selected group (6)

Genetic factors may be of importance for the development of long term diabetic complications Pyke and Tattersall (8) have studied the occurrence of retinopathy in identical twins In twins concordant for diabetes retinopathy is more common and more severe than in twins of whom only one is diabetic Information is lacking about the occurrence of long term complications in diabetics and in those of their children who are diabetic

Most striking concerning the present male patient is the fact that he had a duration of diabetes of 55 years or four years longer than has been reported earlier Except for shin spots (5) he had no signs of long term complications at all

It has been reported that there is no evidence of retinopathy in 25% of patients with a duration of diabetes of 40 years or more (7)

The diabetic daughter in the present study developed minor signs of retinopathy only after 25 years of diabetes and after 36 years this is mainly unchanged

The low degree of long term diabetic complications in both father and daughter is remarkable and so is the fact that the father s diabetes was not well regulated

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EDITORIAL

Necessary Diagnostic Pigeonholes—Defence of Taxonomy in Medicine as well as in Botany

In this number of the Acta we have a paper from Denmark with the title An Arm Chair Study of Diagnostic Decision Making in Gastroenterological Out Patients This paper really contains food for thoughts and the last words in the Abstract possibly hospital records contain too much irrelevant information seem to be a magnificent understatement The readers are specially invited to a discussion of this matter

Many expensive and extensive modern diagnostic methods can only be excused if they give a reliable basis for effective therapy and prognostication by putting the patient s disease in the right pigeonhole Every diagnostic method from simple—but often important—palpation of the pulse or the abdomen to the most elaborate and sophisticated computerized tomography should help us to characterize the disease and from that knowledge find rational treatment Medicine in the future will have to rely also *on simpler methods and choose* between the multitude of tests and investigative procedures preserving what is good and accepting only such innovations as really do yield better information Decision making must be made simpler

It is my feeling that we should try to weed out methods which have been superseded by others that are more effective and not just pile the new ones on the old program Subtraction not only addition is valuable in choosing diagnostic programs The more experienced members of the profession have a great responsibility in this process as they are able to assist the younger in selecting from this abundant flora The French expression Embarras de richesse is really applicable to the present situation For it is clear that the less experienced will feel bewildered A number of laboratory tests and other diagnostic methods that are still in common use should definitely be relegated to medical history

It is also necessary however to adopt new methods The competition between hospitals regarding the introduction of novelties—as soon as possible—may be healthy in itself but may become very expensive The days when an eminent professor of internal medicine in his presidential address at the association meeting in Atlantic City talked about the Two Beckman disease meaning that it was a luxury to have two such expensive gadgets seem to be long passed Yet this happened only 12 years ago and since then the costs of diagnostic machinery have known no limits

A recent problem is the fact that modern patients ask for absolute diagnostic certainty Like the doctor they feel that nothing should be left untried even if there is only the faintest chance that it will add materially to security in diagnosis The mass media inform the public about a new and most expensive technical wonder and the patient of course applies such information to his own problem As doctors we have to remember that there is always a limit to the exactitude of our diagnosis The roentgenologist is well aware of this *when he answers* No signs of instead of No The clinician is asked for a clear yes or no and wants as much confirmation as possible of his judgement The conscientious doctor will therefore arrange a number of diagnostic procedures with this in mind The present trend in many countries to sue the doctor for omitting some diagnostic test automatically leads to the present expensive situation with much over diagnosis—for the doctor s sake! Only when he feels comparatively certain of his judgement will the doctor be able to give his patient a convincing answer Some decades ago a spa advertised Electrocardiographic treatment Many laughed at the naive statement but in reality the method provides many doctors with a good basis for psychological reassurance of an anxious patient So much of our work in decision making is not positively diagnostic but rather negatively designed to help the patient overcome his phobia and doubts about health

Decision making to arrive at a reliable diagnosis usually involves a great many tests The famous

Swiss clinician Sahli coined the saying There is never *one* pathognomonic symptom meaning that a diagnosis is always a puzzle with many bits When quoting this dictum I used to make the exception that the finding of a megaloblastic bone marrow was the same as making the diagnosis of pernicious anemia. We now know that this is no longer true and I guess that Sahli was right in every instance

Many clinicians have become used to accepting the verdict of the anatomical pathologists as final This finality however has a temporary aspect be ing the *last* opinion on the patient after his death when no more facts can be added The importance of death house pathology as a necessary summing up of clinical work cannot be overestimated The situation is different when biopsies are judged Lymphomas are an excellent example of this di lemma. Nothing illustrates the relative value of the morphological analysis better than the rapid change in terminology or classification of these diseases The number size and shape of the pigeonholes have changed rapidly during the last decade It is time that clinicians realize how little value these pigeonholes often have as a basis for treatment and prognostication We frequently find that the same patient gets different diagnoses from consecutive punctures and biopsies The final diagnosis at death [illegible] be a new one The importance of a name is [illegible] in almost all matters between doctor and [illegible] Sometimes I am reminded of an anecdote which my father a professor of orthopaedic sur gery told me many years ago A patient with severe pains in his foot had consulted many doctors with out obtaining any help because nobody knew the exact diagnosis Finally there was one who really understood and told the patient that he had a typical pied douloureux the fact that somebody obvi ously understood made the patient feel ever so much better

Administrators ask for a diagnosis on every pa tient with number and subnumbers in the nomen clature in order to feed the computers that pour out medical statistics Never mind if the input is wrong as long as the machine gets fed Statistics even when beautifully processed mathematically do not improve poor primary data As clinicians we have to realize that the diagnosis consists of many bits that should form a jig saw puzzle and give a clear picture The famous German philosopher Kohler the father of Gestalt psychology advocates the importance of the total picture the Gestalt and anybody who has been active in recognizing plants and making clinical diagnoses must realize the truth of this Linnaeus invented the so-called sexual sys tem which is completely artificial in order to group plants after certain identifiable material and then introduced his famous binominal nomenclature For many species the delimitations are in fact very in distinct and one sometimes wonders whether the old system used by Tournefort with long descrip tions of each plant in Latin terms, was not more correct than trying to squeeze plants from critical species into distinct pigeonholes Still pigeonholes are absolutely necessary The experienced clinician knows a large number of Gestalten each charac terizing a different disease The important thing for the young doctor is to learn and remember as many such pictures as possible with a number of characteristic features in each I learnt how true the idea of the total picture may be when I once tested reading with a patient who suffered from severe aphasia Although this man could not name any of the letters in the alphabet he was able to read out loud even long words when he saw the letters together He saw the Gestalt of the word as we should see the Gestalt of a disease or of a plant if we are botanically minded Botany is an excellent hobby and a good exercise in diagnostic activity for the doctor

Even when we have one well defined etiological factor such as we see it in infections it may be dif ficult to realize that the disease is the same Syph ilis and tuberculosis are excellent examples with symptoms from practically every organ in the body and clinical pictures that may be very confusing. In a talk some time ago Elvin Kabat the well known American immunologist remarked that only the fact that the spirochete had been identified at an early stage could explain all the symptoms in syph ilis as having the same cause Otherwise it would have been quite logical to regard this as a classical autoimmune disease with varied organ manifesta tions and with a specific diagnostic reaction that is a true autoantibody' The antigen used in the Wassermann reaction is a normal body constituent. cardiolipin

Let us return to the discussion about diseases as Gestalten and take lymphoma as an example It is quite clear that the clinical picture differs between the different members of this group whereas the morphological distinctions are much less marked Such clinical data as rapid progression of lymph

gland swelling migration of lymphocytes with increased numbers in the blood (leukemia) fever weight loss secretion of monoclonal globulin in large quantities (macroglobulinemia) splenomegaly (hypersplenism) large bronchial glands (pressure effects) low polyclonal immunoglobulin production (impaired antibody defence) presence or absence of blood lymphocytes covered by or containing Ig all form a picture that is infinitely richer and above all more correct that the morphological patterns There exists one very remarkable study by a professor of pathology at Johns Hopkins University—thus a leader in his speciality—which is very rarely quoted for obvious reasons Still I find it one of the most important studies ever performed in order to test our diagnostic ability He sent the same microscopic preparation to a number of eminent pathologists for diagnosis Sometimes he made different preparations of different parts of the same lymph gland and got different diagnoses The variability of the disease picture is obviously very great and I recently had a patient whose glands were described as showing infiltration of the capsule and of fatty tissue The picture was interpreted as probably Hodgkin s disease This man had a strongly positive Wassermann reaction and was healed with penicillin

I am quite convinced that we should make more such critical tests in order to check the reliability of our findings both regarding percussion (not very highly esteemed any longer) auscultation and palpation A group of radiologists made a similar study regarding X ray pictures of the chest from a very large group of students The pictures were examined by a panel of highly competent doctors but the results were quite unreliable as regards both positive and negative findings

In making decisions we have to remember that a good history is the foundation of all treatment Critical assessment of all findings *together* is necessary and we should never rely too much on diagnostic short cuts Diagnosis is a synthetic process Colleagues interested in these problems should read a book by Henrik R Wulff called Rational Diagnosis and Treatment Blackwell Scientific Publications 1976 ISBN 0 632 00407 X (See also p 149 in this number)

Jan G Waldenstrom

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An Arm-Chair Study of Diagnostic Decision-Making in Gastroenterological Out-Patients

H Friis S W Hansen Vibeke Binder P Riis and H R Wulff

From Medical Department C Herlev University Hospital Herlev Denmark

ABSTRACT It is the purpose of this study to evaluate the efficacy of alternative diagnostic strategies in gastroenterological out patients Photo copies were made of the history and physical exami nation recorded at the first visit of 146 consecutive patients In each case a senior gastroenterologist was asked to state the most likely diagnosis, indicating his or her confidence in the diagnostic bid, and to state which investigations he or she wanted done The diagnostic bids were then compared with the actual diagnoses made at the Out Patient Department The chief result was that the diagnostic bid agreed with the actual diagnosis in 81 % of the 146 cases Agree ment was more frequent in cases where the gastro-enterologist was confident in the diagnostic bid than in those where the prediction was considered less cer tain The high proportion of correct diagnoses sug gests the adoption of a single target strategy in most gastroenterological out patients, i e a strategy aimed at confirming or excluding only that diagnosis which is considered most likely A multi target strategy, taking into account several diagnostic possibilities may be reserved for that minority of cases where the primary diagnostic bid proves incorrect It is sug gested that gastroenterological out patients may be subjected to fewer investigations if these recommen dations are accepted Only 26 % of the items of in formation recorded in the notes were used for the diagnostic prediction, and, possibly, hospital records contain too much irrelevant information

In non acute hospital cases the clinical decision process takes this form first the history of the pa tient is recorded and a full physical examination is made Next various diagnostic possibilities are considered and appropriate investigations are car ried out If the results are considered conclusive a diagnosis is made and when possible treatment is instituted If not other diagnostic possibilities are considered and more investigations are carried out (7) Probably most clinicians will in principle agree with this description but different strategies may be used within this framework Some will at the early stage consider *a number* of likely and less likely diagnoses and consequently they will request a con siderable number of investigations Others will focus on *a single* tentative diagnosis and at first they will only require those investigations which serve to confirm or rule out that particular diagnos tic possibility That strategy saves investigations if the clinician is right in the first place but it is time consuming if he is wrong and subsequently has to consider several other diagnostic possibilities in succession

Such trivial yet important questions of patient management are often discussed and rarely studied but we considered it possible to approach this particular problem simply by studying how fre quently the first diagnostic bid is correct If it is usually correct then the second *single target strategy* is to be preferred whereas the first *multi target strategy* is preferable if the tentative diag nosis is often wrong

In this study a tentative diagnosis was made by one of three senior gastroenterologists solely on the basis of the patient records i e the history and the physical examination which was recorded by the house physician at the patient s first visit to the hospital Therefore the diagnoses were dependent on the accuracy of the information in the notes but at the same time the study provides an opportunity of investigating which items in the notes were con sidered of diagnostic importance

gastroenterologists did not use a single target strategy but tried to guard themselves against diagnostic possibilities which they did not themselves consider probable The possibility of reducing the number of investigations is of course greatest if the tentative diagnosis can be es tablished by positive findings obtained by an X ray examination or endoscopy If the tentative diagnosis is a functional diagnosis which must be made by elimination, the gain is smaller as alterna tive diagnostic possibilities must be ruled out

It was noticeable that only a relatively small amount of the information recorded in the notes was used for the diagnosis That was especially true of the information acquired at the physical examina tion although it must be mentioned that three of the five malignant tumours in the material were diagnosed by recorded physical findings (rectal ex ploration in two patients and abdominal palpation in one) Nevertheless the results raise the question of whether hospital records contain too much irrele vant information and whether part of the routine physical examination could be omitted (e g ex amination of reflexes in all gastroenterological pa tients)

This suggestion may also be viewed in a wider context recently discussed by Campbell (2) He as serts that clinical decision making must be regarded strictly hypothetico-deductive process which s that the decision at each step must be based formulated hypothesis i e a tentative diag nosis Good technique in history taking physical examination and the choice of investigations are dominated by the need to generate hypotheses quickly and to test them critically rather than wast ing time and money collecting information This Popperian attitude is in conflict with some tradi tional views and some new trends in clinical medicine Traditionally we teach medical students and junior doctors to take a full history and to do a complete physical examination before they start thinking about the diagnosis and recently chemical pathologists have suggested that we go even further as regards the collection of routine data They tell us that for technical reasons it is uneconomic just to do those specific tests which are related to the pa tient s particular problem and instead they offer to provide a biochemical profile of all patients The implication of these attitudes is that we should col lect as much data as possible while keeping an open mind and then by induction make a definite or at least a tentative diagnosis Our results suggest that the hypothetico-deductive approach is to be pre ferred and that medical students should be taught accordingly In other studies of diagnostic decision-making in clinical gastroenterology, clinicians and computers have made diagnostic bids and sub sequently the truth of the diagnosis has been established by laparotomy (1 3, 4) In medical gastroenterology such studies cannot be carried out with the same ease, as many of the disease categories are ill defined clinical syndromes which simply describe the patient s clinical picture The truth of such diagnoses cannot be established by independent means (6 7)

In this study we accepted the hospital diagnosis as the final diagnosis and we were not concerned with diagnostic accuracy but with diagnostic strategy Our results are confined to gastroen terological out patients but Hampton et al found in a similar study (5) that the finally accepted diag nosis could be predicted after reading the refer ral letter and taking the history in 83% of 80 un selected medical out patients Therefore it is pos sible that the diagnostic strategy suggested in this paper is also applicable to other categories of medi cal patients

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Accuracy of Arrhythmia Alarms from a Computer-Based Arrhythmia Monitoring System

Johan Hulting and Mats Erik Nygårds

From Department of Medicine I Sodersjukhuset Stockholm and the Department of Medical Information Processing Linkoping University Linkoping Sweden

ABSTRACT A computer based arrhythmia monitoring system has been tested during ten 24-hour periods in a CCU Fifty five patients were monitored for about 1 000 hours A continuous ECG recording from all patients made it possible to estimate the true incidence of arrhythmias Five million heart beats were analyzed The performance of the monitoring system was studied in some defined arrhythmia conditions which were separated into three priority levels *** Alarms included asystole, ventricular fibrillation and ventricular tachycardia ** Alarms comprised consecutive ventricular ectopic beats (VBs), ventricular bigeminy, extreme bradycardia and supraventricular tachycardia * Alarms consisted of tachy/bradycardia, frequent supraventricular or ventricular beats and multiform VBs An arrhythmia alarm persisted on the video screen for at least 4 min (alarm time) A number of monitoring status alarms were also produced by the system The total alarm time for arrhythmias reported by the computer amounted to 122 hours Top priority alarms constituted only 0 9% of total alarm time so that performance in these conditions could not be accurately assessed In the three priority levels computer alarms were present for 76, 74 and 90%, respectively, of the manually determined alarm time Out of detected arrhythmia episodes, a correct alarm message was present for 25 57 and 83% of alarm time in the corresponding priority levels In the whole study the proportion correct to irrelevant alarms was 8 to 1 and a correct diagnosis was present on 3/4 of all write-outs for arrhythmias produced by the automated system

In recent years several computer based arrhythmia monitoring systems have been developed for the coronary care unit (CCU) These systems are intended to improve arrhythmia monitoring and to take over tedious work from the nurses The arrhythmia detector which is the central part of any monitoring system should be properly evaluated and improved if possible before the system can be put into routine use in the CCU Although monitoring systems have been in routine use for some years in several places there have to our knowledge been no reports on their efficacy in the clinical setting

In the present investigation the accuracy of various arrhythmia alarms has been studied during routine monitoring in a CCU A continuous ECG recorded from all patients made it possible to estimate the true incidence of arrhythmias

THE MONITORING SYSTEM

The principles for arrhythmia detection have been described in some detail earlier (1) For the classification of heart beats a scheme for feature extraction has been designed describing each QRST waveform from a number of orthogonal basis signals Based on QRST features waveforms with similar shape are grouped into families and typed as normal or abnormal Ventricular and supraventricular ectopic beats (VBs and SVBs) are separated using both waveform and rhythm data. The power spectrum of the ECG is computed in certain situations and is used to diagnose ventricular fibrillation (VF) and rapid ventricular tachycardia (VT)

Eight patients can be monitored simultaneously In critical alarm situations the nurse is alerted by a wireless pocket receiver activated by the computer All alarms are displayed on video screens located in the central station and in the patient rooms The event which caused the alarm is documented by an automatic ECG write-out on a two-channel mingograph presenting a delayed bipolar ECG patient room time of the event and the arrhythmia diagnosis in α-numeric form The heart rate (HR) and the number of VBs are stored in the computer for the last 24 hours Alarm messages remain in storage for the same

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Table I *Arrhythmia and monitoring status alarms given by the monitoring system*

VF=ventricular fibrillation VT=ventricular tachycardia VB=ventricular ectopic beat SV=supraventricular

Alarm no	Priority Level	Priority N	Reset time (min)	Type of alarm
1	***	1	20	Asystole
2	***	1	20*	VF
3	***	2	20*	VT
4	**	5	4	More than 3 consecutive VBs
5	**	6	4	Idioventricular rhythm
6	**	7	4	Bradycardia
7	**	8	4	2–3 consecutive VBs
8	**	9	4	Ventricular bigeminy
9	**	10	4	SV tachycardia
10	*	14	4	More than 5 VBs/min
11	*	15	4	Multiform VBs
12	*	16	4	More than 5 SV beats/min
13	*	17	4	Bradycardia
14	*	17	4	Tachycardia
15	**	3	1	What has happened?
16	**	4	1	Check electrodes!
17	**	11	1	New QRS
18	*	12	1	Artifacts in the ECG
19	*	12	1	Unfavorable placement of electrodes
20	*	13	1	Low amplitude
21	*	18	1	Faulty electrode contact

* If the cause of alarm no longer persists the continuous alert signal will be turned off after 10 sec However the alarm message will remain on the screen if not overridden a new high priority alarm

or until the alarm buffer is full Display of stored may be requested from a numeric keyboards located in the patient rooms and the nurses station Also a number of other interactive procedures such as altering the alarm limits are available The system uses a 16-bit general purpose minicomputer (Datasaab D5/30) with 28 K words of core memory The programs are written in Basic FORTRAN and assembly language

The alarm conditions studied are listed in Table I Concerning the presentation of alarm signals the conditions are separated into three main priority groups the highest priority messages (***) being accompanied by a flashing red light and a continuous audible alarm whereas the two-star (**) alarms are presented with a steady red light and a short tone and the low priority messages (*) are presented on the video screen only

All alarms are ranked according to priority and the detection of an alarm condition with higher or equal ranking than a present alarm results in a new message on the video screen. The alarm is automatically reset if the condition has been absent for a certain time (Table I) However alarm reset may also be done manually As shown in Table I, the presence of some artifact alarms implies blocking of the VB detection

For the purpose of this study *all* alarms produced by the monitoring system were automatically punched on paper tape From these tapes lists were produced one for each patient which summarized the results of the computer analysis

Average HR and the number of SVBs and VBs in each patient were also punched every 10 min and this made it possible to estimate the number of different beats analyzed by the computer system during the study

STUDY BASE AND METHODS

The monitoring system has been in routine use at the CCU at Sodersjukhuset Stockholm since the beginning of 1976 In Aug 1976 a comparison was performed between alarms generated by the computer and alarm situations detected by a physician after scrutiny of a continuous bipolar ECG record (10 mm/sec) One channel of an 8-channel mingograph (Elema Schonander 62) was reserved for an α numeric time print-out generated by the computer Up to seven patients could be studied simultaneously Recordings were made every third day during one month i e for ten 24-hour periods and usually started and ended at 10 a m The patients were studied only on the first occasion in the CCU and not for more than 24 hours A conventional HR alarm system operated simultaneously with the computer analysis and the same HR limits for alarms were used in the two systems HR alarms from the conventional monitoring system produced markings on the continuous ECG record and the number and probable reasons for these alarms were studied in order to make a rough comparison between the two systems With the conventional system only one alarm each minute was counted even when the alarm was triggered several times during 1 min for the same patient In the above mentioned comparison all alarm write-outs from the two-channel mingograph were counted and classified solely on the basis of the computer diagnosis These write-outs were also necessary in order to establish an absolute relationship between an alarm and an arrhythmia episode

The study did not influence routine patient care or treatment Routine 12 lead diagnostic ECGs were recorded before and after the 24-hour period Lidocaine was given to patients with acute myocardial infarction (AMI) less than 48 hours old when VT or R on T VBs were detected by the nurse with or without the aid of the monitoring system Most patients in this study were admitted to the CCU because of a suspicion of AMI This diagnosis was confirmed in 23 out of a total of 55 In 14 other patients an arrhythmia diagnosis was made and in another 5 patients no significant heart disease was found In the patients with AMI the time from onset of symptoms to the start of the continuous ECG recording varied between 5 and 90 hours (mean 31) Day 1 is excluded from these figures Lidocaine had been given to three patients before the study and two others received this drug during the 24-hour period that was investigated Two patients died during the recording period In neither however was VF observed

The ECG recordings were analyzed beat by beat by a physician who had no information about the results from the computer analysis Abnormal beats were classified as follows *V*=premature ventricular beat *P*=possible ven-

tricular beat N=abnormal supraventricular beat S=premature beat with normal QRST shape The diagnosis of abnormal beats was based on subjective criteria using prematurity QRST aberrancy and increase in QRS interval A deviation from normal in all three respects was set up as a requirement for a V beat A deviation from normal in two respects resulted in a P diagnosis However narrow premature and slightly aberrant beats were usually classified as N In the event of uncertainty whether to refer a complex to the P or N class the former alternative was chosen The P wave in the ECG was also used in the classification of complexes when possible Runs of widened beats with abnormal morphology and unchanged P–Q and R–R intervals (intermittent bundle branch block) were classified as N This group comprised abnormal beats not fulfilling the V or P criteria Abnormal beats disturbed by artifacts were classified as V P N or S whereas deranged ordinary complexes were classified as artifacts In the results P and V beats were combined in to a VB category correspondingly N and S beats were not separated but regarded as SVBs

Based on the physician s classification of beats which was considered correct the highest priority alarm that should have been presented with an ideal performance of the monitoring system was determined for each minute of monitoring in each patient The actual alarms given by the system were then compared minute by minute with the expected outcome at ideal performance The result of the comparison was summarized in terms of total time for correct alarms (C) lower (L) or higher (H) priority than expected alarm missed alarms (U) irrelevant (false positive) alarms (I) and monitoring status alarms possibly suppressing the detection of a true arrhythmia episode (A) The number of minutes in each of the above categories was calculated for each of the arrhythmia alarms (nos 1–16) with reference except for I alarms to the physician s interpretation i e L and H alarms were referred to the expected alarm type not to the actual alarm Monitoring status alarms (nos 15–21) were only studied in situations when the presence of the alarm was directly related to the actual arrhythmia episode (alarm nos 15 and 17) when the actual alarm possibly suppressed the detection of the true event If a single alarm was manually reset in a shorter time than 4 min this interval was still added to the total alarm time If an alarm lasting for several minutes was reset earlier than expected the time remaining to correct alarm reset was regarded as U For U alarms a 4-minute reset time was counted H alarm was noted only if the reported alarm has been elicited by the true alarm event In other cases one I and one U alarm were marked An L alarm was notified only if the given alarm could be related to the real ECG event otherwise a U alarm was noted I alarms with a lower priority than the highest priority alarm were ignored A C alarm was not noted in U A or L situations even if the reported alarm in itself was correct Alarm 12 when undetected was marked as L if the rhythm at the nearest 10 min print-out was noted as irregular

Arrhythmias that were detected by the computer system but overlooked by the physician are not included in the results Alarm time for these conditions will be briefly reported in the discussion

The alarm function for tachycardia bradycardia and multiform VBs was studied only when such an alarm was produced by the system For the diagnosis of VT or supraventricular tachycardia (SVT) at least 4 consecutive abnormal beats were required The lower HR limit for tachycardia regarding VT SVT or normal rhythm was set at 120 beats/min For the bradycardia alarm the upper limit was set routinely at 50 and this limit as well as the tachycardia limit for normal rhythm could be chosen manually Alarm 6 was given when HR fell more than 15 beats/min below the bradycardia limit The HR alarm limits were selected so as not to exaggerate the number of HR alarms and these limits were modified when there was an apparent change in HR deviating outside the preset rate limits Bigeminy was defined as 3 VBs alternating with normal complexes Further definition and explanation of the alarms is given elsewhere (4) VB detection was inhibited for 1 min at all artifact alarms except no 21 Alarms were not studied in situations when the continuous ECG recording was interrupted e g during paper chart change or ink bottle replacement

RESULTS

Fifty five patients were monitored during 10 days for a total of 1 009 hours The continuous ECG recordings were interrupted for nearly 2 hours and since 5 5 patients were studied on the average about 10 hours of monitoring was omitted from the study Monitoring time in individual patients varied between 0 2 and 24 0 hours (mean 18 3) Twenty five patients were monitored for 24 hours The total number of ECG complexes analyzed was $5\ 0 \times 10^7$ The number of various beats detected on each day of the study is given in Table II which also shows the number of computer alarms presented on the mingograph as well as the number of alarms generated by the conventional HR dependent alarm system About 1% of all beats were considered abnormal by the computer with a moderate predominance for SVBs There were wide variations in the number of abnormal complexes between the different days In 19 patients with on the average less than 1 VB/hour detected in the computer analysis a total of 153 VBs were detected during 415 hours of monitoring Manual classification of these patients also showed a very low true VB frequency

The number of alarm write-outs generated by the computer system was more than 5 times as great as the number of alarms from the conventional monitoring system which however did not distinguish between arrhythmias and artifacts Half of the alarms given by the conventional system were caused by artifacts the rest by various arrhythmias

Table II *ECG material and number of alarms given by the computer and the conventional monitoring system*

VBs=ventricular ectopic beats SVBs=supraventricular ectopic beats

Day	No of pats	Monitored time (h)	No of beats ×10⁻³	Computer detection		No of computer alarms for arrhythmias/ artifacts	No of alarms from the HR dependent alarm system
				VBs	SVBs		
1	6	102 0	570 7	974	3 723	108/127	18
2	7	135 1	728 4	5 713	7 943	206/101	74
3	6	129 0	624 9	878	6 524	130/ 44	17
4	6	77 1	347 4	394	1 229	43/ 36	18
5	6	93 7	398 2	1 097	175	79/ 35	49
6	5	99 6	442 3	1 092	1 498	54/159	25
7	4	73 0	308 0	42	261	13/ 79	8
8	6	136 9	771 5	3 156	2 482	128/153	62
9	5	98 2	441 4	115	179	7/ 20	4
10	4	64 2	337 7	4 878	4 194	139/ 30	61
Total	55	1 008 8	4 970 5	18 339	28 208	907/784	336

of which true tachy or bradycardia constituted about 60% As to the alarm write outs from the computer system lowest priority alarms constituted 75 and 71% of the total number of alarms for arrhythmias and artifacts respectively

The performance of the computer based monitoring system in the arrhythmia alarm conditions is summarized in Table III The highest priority alarm conditions occurred infrequently

Asystole was seen once and was reported as what has happened?' due to the detection of baseline shifts in the ECG VF was falsely reported in one patient with artifacts superimposed on an essentially normal ECG tracing VT was correctly

Table III *Results from the computer analysis of various arrhythmias*

rrect alarm *L*=lower priority than actual event *U*=undetected event *H*=higher priority than actual event evant detection *A*=rhythm analysis inhibited by artifacts VF=ventricular fibrillation VT=ventricular a VB=ventricular ectopic beat SV=supraventricular

Priority level	Arrhythmia	Alarm time (min)						No of alarms	
		C	*L*	*U*	*H*	*I*	*A*	Correct	Irrelevant
***	Asystole		9						
	VF					10			1
	VT	8	11	10		24	4	2	5
Total		8	20	10		34	4	2	6
**	More than 3 consecutive VBs	8	8			14		2	4
	Bradycardia	2	4					1	
	2 or 3 consecutive VBs	401	251	258	4	91		75	21
	Ventricular bigeminy	396	220	215	4		42	74	
	SV tachycardia	124	62	51		4	10	27	1
Total		931	545	524	8	109	52	179	26
*	More than 5 VBs/min	1 157	407	232	2	35	4	156	9
	Multiform VBs	285			24	77		60	17
	More than 5 SV beats/min	1 439	114	260	181	105		191	27
	Bradycardia	819				6		77	2
	Tachycardia	991						67	
Total		4 691	521	492	207	223	4	551	55
Total arrhythmia alarms		5 630	1 086	1 026	215	366	60	732	87

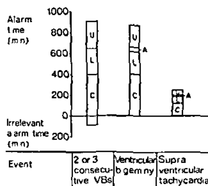


Fig 1 Monitoring system performance in some arrhythmias of the second highest priority level *VB*=ventricular ectopic beat *C*=correct diagnosis *U*=undetected event *L*=lower priority than correct (even if within correct priority level) *A* artifact alarm present

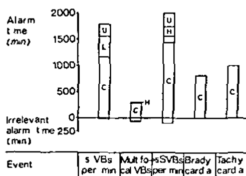


Fig 2 Monitoring system performance for arrhythmias in the lowest priority level *SVB*=supraventricular ectopic beat *H*=higher priority than correct (even if within the same priority level) Other symbols as in Fig 1

diagnosed on 2 occasions undetected on 2 and falsely reported on 5 As to the two-star alarm conditions episodes of more than 3 consecutive VBs and marked bradycardia were rare and thus performance in these arrhythmias cannot be properly evaluated The results of the computer monitoring in other second level alarm conditions are presented in Fig 1 Alarms for 2 or 3 consecutive VBs ventricular bigeminy and SVT were correctly reported in about 50% of the expected alarm time at optimal performance Irrelevant alarms for consecutive VBs amounted to 20% of the total alarm time in this condition Irrelevant alarms for ventricular bigeminy or SVT were virtually absent The lowest priority arrhythmia alarms were present for 78 hours or 8% of the total monitoring time The results are presented in Fig 2 The alarm for more than 5 SVBs/min was more common than the corresponding alarm for frequent VBs The alarm time for undetected arrhythmias was about the same in these two conditions and the number of *I* alarms was small in relation to the total Frequent SVBs were reported as irregular rhythm for a total of about 2 hours False positive SVB detection in irregular rhythm resulted in a more than 5 SVBs/min alarm for 3 hours

L and *U* alarms in multiform VBs tachycardia and low priority bradycardia were not studied Alarm time for irrelevant arrhythmia detection was brief in those conditions

The total alarm time ($C+L+H+U+A$) at optimal performance for arrhythmias was 134 hours for

Table IV *Monitoring status alarms given during 1 009 hours of arrhythmia monitoring*
Figures within parentheses indicate no of patients contributing to the preceding figure

Day	No of pats	No of alarms: New QRS	No of alarms: What has happened?	No of alarms: Check electrodes	Alarm time (min): Artifacts in the ECG	Alarm time (min): Unfavorable placement of electrodes	Alarm time (min): Low amplitude	Alarm time (min): Faulty electrode contact
1	6	7 (4)	13 (2)	8 (3)	7 (2)	33 (3)	0	274 (2)
2	7	4 (2)	37 (3)	11 (4)	7 (3)	61 (2)	20 (2)	11 (2)
3	6	4 (1)	1 (1)	3 (3)	12 (3)	13 (3)	5 (2)	14 (3)
4	6	0	0	2 (1)	5 (2)	81 (2)	0	29 (2)
5	6	4 (3)	6 (3)	4 (3)	1 (1)	32 (2)	31 (1)	3 (3)
6	5	7 (4)	8 (3)	21 (5)	9 (3)	735 (2)	363 (3)	107 (4)
7	4	24 (3)	4 (2)	5 (3)	1 (1)	75 (2)	143 (2)	19 (2)
8	6	4 (3)	6 (3)	21 (5)	15 (3)	325 (4)	3 (2)	13 (4)
9	5	10 (1)	2 (2)	3 (3)	0	3 (2)	50 (2)	1 (1)
10	4	3 (1)	1 (1)	15 (3)	4 (2)	1 (1)	0	8 (2)
Total	55	67 (22)	78 (20)	93 (33)	61 (20)	1 359 (23)	615 (14)	479 (25)

70% of this time a *C* alarm was present for 13% an *L* alarm for 3% an *H* alarm for 13% *U* alarm and for 0 7% of the total time the arrhythmia occurred simultaneously with a monitoring status alarm of a higher priority The total number of *I* alarms was 89 compared to 732 for the *C* alarms An *I* alarm was present for 6 1 hours, i e for 5% of the total arrhythmia alarm time

Table IV shows the monitoring status alarms given each day of the study Monitoring status alarms on the second priority level occurred with an average frequency between 0 07 and 0 09 per patient hour The lowest priority monitoring status alarms are presented in alarm time in Table IV The alarm indicating artifacts in the ECG was reported for only 2 7 hours whereas unfavorable placement of electrodes occurred for 22 7 hours or 2 3% of total monitoring time Low amplitude and faulty electrode contact were reported rather frequently or for 1 8% of total monitoring time It is apparent from Table IV that each type of monitoring status alarm was only reported in a minority of the patients In 9 patients monitored for 122 hours no monitoring status alarms at all were produced

DISCUSSION

In this study the performance of an automated ythmia monitoring system has been studied dur n 24-hour periods in a CCU The selection of ents for the study was not influenced by CCU personnel Out of a total of 55 patients 22 (40%) proved to have AMI

In order to obtain clinically relevant results it was decided not to study the beat by beat performance which would be practically impossible in a material of this size but to study the performance in some special alarm situations (Table II) Some of the arrhythmias (nos 4 7 8 10 and 11) have been considered important forerunners of serious arrhythmias in AMI (2) Apart from previously mentioned arrhythmias the monitoring system has been designed to make another two arrhythmia statements namely R on T VB and missing QRS The results of the computer analysis in these two conditions were not studied in detail It was apparent that the human observer overlooked several incidences of the R on T phenomenon also this condition sometimes caused confusion because VBs occurring on the final portion of the preceding T wave are relatively common The R on T alarm was reported on 18 occasions in 4 patients most of these alarms were true Missing QRSs were usually overlooked by the computer due to an error in the computer algorithm

The arrhythmias in the various priority levels were chosen arbitrarily on the basis of their probable clinical relevance A similar philosophy for the presentation of alarms has been reported by Sanders et al (5)

The performance of the alarm function is presented as alarm time rather than the number of alarms of various categories In our opinion this makes it possible to get a better view of the influence of automated monitoring on CCU staff The results in continuous arrhythmia conditions such as tachy or bradycardia could be treated uniformly with distinct alarm events e g paired VBs Also performance in the highest priority arrhythmias will be emphasized whereas the method reduces alarm time for sporadic artifacts of lowest priority since alarm reset time for these alarms was set at 1 min compared to 4 min for the arrhythmia alarms The total number of correct alarms in relation to the total number of arrhythmia alarms was 0 81 based on alarm time the corresponding figure was about the same or 0 77 Thus there is probably little difference between the above two principles for estimating monitoring system performance in various arrhythmias As monitoring time in the study was maximized to 24 hours patients treated in the unit for several days did not necessarily influence the results more than patients observed for only one or two days The actual total number of patients in the unit during the 10-day period was 71 and total monitoring time for these was 1 207 hours

Some arrhythmia episodes overlooked by the physician were detected by the automated system Total alarm time for these events was 2 6 hours and 77% resulted from undetected SVBs and short runs of SVT Thus at least 2% of true alarm time was not notified in the manual interpretation

Somewhat unexpectedly the number of alarms from the computer system was 5 times as great as the number of alarms from the conventional system and despite the use of different methods for counting alarms a marked difference between the two systems was indicated The proportion of alarms resulting from arrhythmias and artifacts was about the same with the two systems The computer system gave alarms to indicate special electrode problems and it is likely that these alarms also con-

tributed to reduce the number of artifact alarms from the conventional system Most monitoring status alarms from this system resulted from dis connection of ECG cables from the patients Thus our HR-dependent alarm system was found to be relatively insensitive to muscle artifacts and other common minor disturbances of the ECG signal

It was obvious that the staff did not always im mediately follow the advice given by the computer regarding the electrodes since alarms 18–21 were reported repeatedly in some patients In one patient with low voltage in the ECG a suitable electrode position could not be obtained and alarm 20 was present for 12 hours In all other patients an accept able QRST morphology was obtained Special prob-lems however arose in one patient with a pacemaker which operated intermittently during the study and gave rise to several more than 3 con secutive VBs alarms This diagnosis was consid ered correct On the other hand if pacemaker rhythm was considered normal resumption of spontaneous activity resulted in a new QRS alarm

The low incidence of *** alarm conditions in this study makes it impossible to evaluate the computer performance in these conditions accurately In this study only 3 of 22 patients with AMI had VT There are two important reasons for the low VT inci dence Firstly the time from onset of symptoms to the start of the recording was relatively long av eraging 31 hours and was less than 12 hours in only 2 cases Using a continuous ECG recording for up to 24 hours in AMI Mogensen (3) found a VT incidence of 65% compared with 12% in a control group Secondly in most other recently published studies VT has been defined as 3 or more consecu tive VBs with a frequency above 100 and in our study another 2 patients with AMI had VT with this definition

The diagnostic criteria used for manual VB diagnosis in this study may result in a slight over estimation of ventricular relative to supraventricu lar arrhythmias Total *L* and *U* alarm time was reduced by a factor of 0 7 if suspected VBs (*P* complexes) were regarded as true SVBs when not interpreted as VBs by the computer

In a previous study we tested the performance of our system using essentially the same algorithms for the arrhythmia analysis (1) Taped ECG material was fed into the computer and electrode malfunc tioning and other disturbances could not be cor rected during the analysis In that study 3 6 artifact alarms per patient hour were given and arrhythmia analysis was blocked for 8% of total time com pared with about 4% in the present study The latter figure however does not include time for inhibition of arrhythmia analysis due to brief artifacts not resulting in an artifact alarm This time was less than 1% of the total monitoring time

Unfavorable placement of electrodes was the most common artifact alarm in the present study but was not included in the earlier version of the system It thus appears that artifact inhibition time was at least reduced by 50% when electrode malfunction could be corrected

Vetter and Julian (6) using a special hybrid com puter reported very high figures for the detection of various arrhythmias These however were not specifically classified by the monitoring system Results from routine long term monitoring have not been reported for other monitoring systems

The low percentage of false alarms has certainly facilitated the acceptance of our system among the nurses Although the system will probably improve arrhythmia monitoring much still remains to be learned about the most relevant arrhythmias or ECG events to monitor and the prognostic value of various arrhythmias but it is our hope that computer assisted monitoring will promote such progress

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Auricular Tyrosine Hydroxylase and Dopamine-β-Hydroxylase Activities and Noradrenaline Content in Ischaemic Heart Disease

O Penttila E Merikallio J Pispa E Klinge
P Siltanen and K Kyosola

From the Departments of Pharmacology and Medical Chemistry University of Helsinki and the Department of Thoracic Surgery and the Cardiovascular Laboratory First Department of Medicine University Central Hospital Helsinki Finland

ABSTRACT Assessments were made of the tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) activities as well as the noradrenaline (NA) content of samples excised from right auricular tissue during cardiac surgery on a total of 55 patients with ischaemic heart disease (IHD) valvular heart disease (VHD), uncomplicated atrial septal defect (ASD) or congestive heart failure (CHF) The NA content was significantly higher in the IHD group than in the other three groups The TH activity was highest in the IHD group although the difference was statistically significant only compared with the ASD and CHF groups The DBH activity was also highest in the IHD group but again the difference was statistically significant only compared with the ASD and CHF groups In the whole material there was a significant positive correlation between the NA content and TH or DBH activity, as well as between TH and DBH activity In the IHD group there was a significant positive correlation between heart volume and TH activity The results suggest that at least compared with ASD and CHF the sympathetic tone is relatively high in IHD possibly involving an enhanced NA turnover

There is increasing evidence that the activities of the biosynthetic enzymes are important for regulating the content of noradrenaline (NA) in tissue (11 21) Tyrosine hydroxylase (TH) (tyrosine 3 monooxygenase EC 1 14 16 2) is considered to be the rate limiting step in the synthesis of NA (4 13) In human congestive heart failure (CHF) it has been shown that the myocardial TH activity is reduced (9) and there is also a marked myocardial NA depletion (6) *In contrast to the depletion of NA in CHF* the auricular myocardial NA content is relatively high in ischaemic heart disease (IHD) (18)

The aim of the present study was to estimate as an indicator of sympathetic nerve tonicity whether the relatively high auricular NA content observed in IHD is associated with high auricular activity of TH and/or dopamine β hydroxylase (DBH) (dopamine β-monooxygenase EC 1 14 17 1) and to compare these activites with those in patients with uni or multivalvular heart disease (VHD) and uncomplicated atrial septal defect (ASD) with or without CHF For obvious reasons no normal control material was available

PATIENTS AND METHODS

Classification of the patients

Samples of the right atrial appendage were obtained during coronary bypass operation from 19 patients suffering from IHD Their ages ranged from 26 to 58 years and three of them were females They were selected for the operation on the basis of anamnestic clinical and coronary angiographic data The second group consisted of 10 patients 3 females and 7 males with VHD without clinically overt congestive myocardial failure Their ages ranged from 30 to 62 years Several patients had multivalvular disease but the predominant lesions were mitral stenosis in 3 mitral regurgitation in 2 aortic stenosis in 1 and aortic regurgitation in 4 patients third group consisted of 14 patients 10 femal males with uncomplicated ASD of secundum

Reprint requests to O Penttila M D Department of Pharmacology University of Helsinki Siltavuorenpenger 10 SF-00170 Helsinki 17 Finland

Table I *Clinical and laboratory data and atrial noradrenaline (NA) content and tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) activities in the four patient groups (mean ± S D)*

IHD=ischaemic heart disease VHD=valvular heart disease ASD=atrial septal defect CHF=congestive heart failure

	N	Age (y)	Heart volume (cm³/m²)	BP (mmHg)	NA (μg/g)	TH (nmoles ×g⁻¹×min⁻¹)	DBH (nmoles ×g⁻¹×min⁻¹)
IHD	19	44 3	462	141/92	2 51	0 48	2 33
		7 8	72	16 10	0 71	0 16	0 92
VHD	10	46 9	787[a]	133/72[b]	1 78[c]	0 41	1 93
		10 4	198	13 19	0 79	0 21	0 96
ASD	14	41 6	567[a]	134/82[b]	1 33[c]	0 30[d]	1 46
		13 1	73	24 13	0 70	0 15	0 90
CHF	12	48 7	970	130/73[b]	0 64[c]	0 19[d]	0 90[e]
		7 0	207	28 18	0 34	0 11	0 58

[a] Significantly higher than in the IHD group ($p<0\ 001$)
[b] Significantly lower than diastolic BP in the IHD group (VHD/IHD and CHF/IHD $p<0\ 001$ ASD/IHD $p<0\ 02$)
[c] Significantly lower than in the IHD group (VHD/IHD $p<0\ 02$ ASD/IHD and CHF/IHD $p<0\ 001$)
[d] Significantly lower than in the IHD group (ASD/IHD $p<0\ 01$ CHF/IHD $p<0\ 001$)
[e] Significantly lower than in the IHD group (ASD/IHD $p<0\ 02$ CHF/IHD $p<0\ 001$)

ages ranged from 18 to 59 years The fourth group consisted of 12 patients 2 females and 10 males who had experienced CHF prior to surgery based either on ASD in 2 or VHD in 10 patients Their ages ranged from 30 to 55 years The degree of myocardial failure was assessed according to the clinical and laboratory findings and the classification of the New York Heart Association (16) A summary of some clinical and laboratory data on the patient groups is presented in Table I

treatment and anaesthesia

usual daily medication with digitalis diuretics anti hmic and antihypertensive drugs anticoagulants clofibrate and nitroglycerin was continued up to the operation with the exception of the β-adrenergic blocking drugs which were withdrawn seven days before No drugs known to interfere with the tissue NA content were used

Standard premedication (atropine and pethidine) and anaesthesia (thiopentone sodium oxygen+nitrous oxide pethidine or anilendine alcuronium or pancuronium) were employed The biopsies from a standard auricular tissue site were excised before institution of cardiopulmonary bypass and operative correction

Determination of enzyme activities and NA content

Tissue samples weighing 100–200 mg for determination of enzyme activities were put immediately after biopsy into liquid nitrogen and stored at −70°C until all the material had been collected The TH activity was assayed according to Waymire et al (22) without any major modification The DBH activity was measured according to Molinoff et al (14) except that the pH at the first incubation was 5 0

For the quantitative determination of NA a spectrophotofluorimetric method (3) was used in a slightly modified form (12) Tissue samples weighing 50–150 mg were immediately cooled and kept in ice until treated further less than 4 hours after biopsy

In addition the heart volume (cm³/m²) and the systemic resting BP (mmHg) were recorded during the week before the operation

RESULTS

In the IHD group the mean NA content (μg/g±S D wet tissue) was 2 51±0 71 and in the VHD group 1 78±0 79 The difference is statistically significant ($p<0\ 02$) The values in the ASD and CHF groups were 1 33±0 70 and 0 64±0 34 respectively both significantly lower than that in the IHD group ($p<0\ 001$) (Table I) The NA content in the CHF group is significantly lower than in the ASD and VHD groups (ASD/CHF $p<0\ 01$ VHD/CHF $p<0\ 001$) There is no statistically significant difference between the ASD and VHD groups ($p<0\ 2$)

In the IHD group the mean TH activity (nmoles ×g⁻¹×min⁻¹±S D per wet weight of tissue) was 0 48±0 16 and in the VHD group 0 41±0 21 The difference is not statistically significant ($p<0\ 3$) Compared with the IHD group the TH activities were significantly lower in both the ASD and the CHF group being 0 30±0 15 ($p<0\ 01$) and 0 19±0 11 ($p<0\ 001$) respectively (Table I) The TH activity is significantly lower in the CHF group than in the VHD ($p<0\ 01$) and ASD groups ($p<0\ 05$) The difference between the ASD and VHD groups is not statistically significant ($p<0\ 2$)

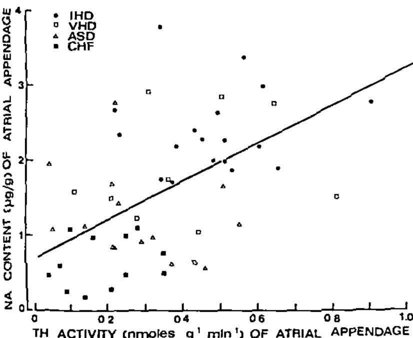


Fig 1 Relationship between tyrosine hydroxylase (*TH*) activity and noradrenaline (NA) content in right auricular samples excised from patients with ischaemic heart disease (*IHD* $n=19$) valvular heart disease (*VHD* $n=10$) uncomplicated atrial septal defect (*ASD* $n=14$) or congestive heart failure (*CHF* $n=12$) The correlation is statistically significant ($r=0.53$ $p<0.001$ $n=55$)

In the IHD group the mean DBH activity (nmoles×g⁻¹×min⁻¹±S D per wet weight of tissue) was 2 33±0 92 and in the VHD group 1 93 ±0 96 The difference is not statistically significant ($p<0.3$) Compared with the IHD group the DBH activities were significantly lower in both the ASD and the CHF groups being 1 46±0 90 ($p<0.02$) and 0 90±0 58 ($p<0.001$) respectively (Table I) The DBH activity is significantly lower in the CHF group than in the VHD group ($p<0.01$) but not significantly lower than in the ASD group ($p<0.1$) Neither is the difference between the

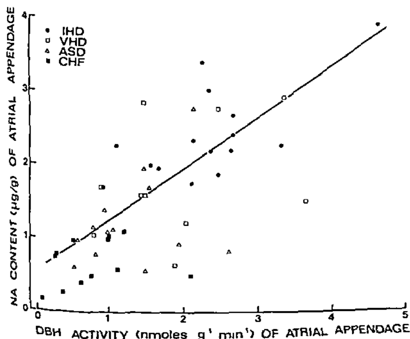


Fig 2 Relationship between dopamine β-hydroxylase (*DBH*) activity and noradrenaline (*NA*) content in the same samples as in Fig 1 The correlation is statistically significant ($r=0.66$ $p<0.001$ $n=55$)

Table II *Correlation between heart volume (HV) and NA content TH or DBH activities of right atrial tissue in the whole study population and in the patient groups IHD VHD ASD and CHF*

Abbreviations as in Table I

Groups	*N*	Coefficient of correla tion	Significance
HV/NA			
Total	55	−0 56	$p<0\ 001$
IHD	19	+0 25	NS
VHD	10	−0 39	NS
ASD	14	−0 44	NS
CHF	12	−0 10	NS
HV/TH			
Total	55	−0 42	$p<0\ 01$
IHD	19	+0 60	$p<0\ 01$
VHD	10	−0 66	$p<0\ 05$
ASD	14	−0 18	NS
CHF	12	−0 22	NS
HV/DBH			
Total	55	−0 42	$p<0\ 01$
IHD	19	+0 17	NS
VHD	10	−0 20	NS
ASD	14	−0 07	NS
CHF	12	−0 38	NS

NS = not significant

ASD and VHD groups statistically significant ($p<0\ 3$)

There is a significant positive correlation be tween NA content and TH activity in atrial tis sue specimens of the whole material ($r=0\ 53$ $p<0\ 001$ $n=55$) (Fig 1) A corresponding signifi cant positive correlation is also found in this material between NA content and DBH activity ($r=0\ 66$ $p<0\ 001$ $n=55$) (Fig 2) These correla tions were non significant within all the individual patient groups On the other hand in the total material there is a significant correlation between TH and DBH activities ($r=0\ 36$ $p<0\ 01$ $n=55$) No significant correlation exists between either the enzyme activities or the NA content and the systemic diastolic BP

The correlation between heart volume and either NA *content or TH or DBH activity is presented in* Table II In the whole material there is a signifi cant inverse correlation between heart volume and each of these three parameters Within the individ ual patient groups there is in the IHD group a significant positive correlation between heart volume and TH activity ($p<0\ 01$) In the VHD group the same parameters show a significant in verse correlation ($p<0\ 05$) No other significant correlations were found

DISCUSSION

It now appears that about 90% of the NA present in the heart is synthetized locally (5) The rate of synthesis of NA in peripheral neurones is regulated by neuronal activity (11) The activity of TH has been observed to decrease markedly in the failing heart of both animals and man (9 19) Our results confirm the earlier observations concerning low TH activity in CHF There was also a significant positive correlation between NA content and TH activity as shown earlier by DeQuattro et al (9) In the present study also the activity of DBH was low in CHF In addition, in the whole population under study a significant correlation existed be tween NA content and DBH activity as well as between TH and DBH activities These results indicate that the reduction in the activities of both biosynthetic enzymes contributes to the depletion of the adrenergic neurotransmitter in CHF The actual cause of this reduction has not yet been found These results also confirm our earlier find ings on the auricular NA content in various heart diseases (18) except for the present significant dif ference between the IHD and VHD groups How ever this may have to do with the somewhat arbitrary clinical classification of valvular patients concerning the degree or absence of CHF

The moderate although statistically significant difference between the NA contents in the IHD and VHD groups possibly accounts for the insignifi cant differences in enzyme activities of the same groups On the other hand the TH and DBH activities were significantly lower in the ASD and CHF groups than in the IHD group, which is in accordance with the much lower NA content in these groups As evaluated from the neurotrans mitter content and the activities of the biosynthetic enzymes it appears that at least compared with uncomplicated ASD and CHF the local sympathet ic tone in IHD is relatively high possibly involving an increased turnover rate of NA Owing to the lack of a normal control material however it can not be stated that the sympathetic activity is elevated in IHD

There was in the whole population an inverse correlation between heart volume and NA content or the enzyme activities The hypertrophy of the

myocardial muscle mass as well as the increase in fibrosis due to degenerative processes in diseased hearts very likely contribute to the decreased NA content and TH activity observed in myocardial failure both in experimental conditions and in man However the reduction in NA content and plausibly also in *TH* activity cannot be explained solely in terms of simple dilution of adrenergic nerve endings (7, 8 9) Studies by a histochemical method in corresponding patient groups (17) as well as the significant positive correlation found in this study in the *IHD* group between heart volume and TH activity further support this concept

The reason for the significant positive correlation found between heart volume and TH activity in the present IHD group remains to be established Nevertheless this finding suggests that in IHD prior to possible manifestation of myocardial failure the activity of the rate limiting enzyme of NA biosynthesis correlates positively to increasing heart volume

Recently it has been postulated by Mudge et al (15) that the adrenergically mediated coronary vasoconstriction must also be considered as a possible pathogenetic mechanism for myocardial ischaemia in patients with coronary disease They were able to show that the cold pressor test increased coronary vascular resistance in IHD and concluded that this was consistent with the hypothesis that the tone of coronary vasculature is actively regulated by α adrenergic receptors It has also been shown that central sympathetic stimulation in dogs results in direct coronary vasoconstriction mediated by activation of α adrenergic receptors (2 10) Both the relatively high auricular—and probably also ventricular—NA content in the *IHD* group and the dense perivascular and parenchymatous adrenergic nerve net shown by fluorescence microscopy in IHD (17) indicate that there are good local conditions for promoting adrenergic tone resulting in constriction of small myocardial resistance vessels

It can be considered that these results indirectly support the hypothesis of Raab (20) concerning the significance of catecholamines in the development of angina pectoris and heart infarction as well as the hypothesis introduced by Baroldi (1) that in some cases of sudden death histological and pathological changes in myocardium strongly suggest the occurrence of a primary catecholamine like death

ACKNOWLEDGEMENT

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Diagnostic Value for Acute Myocardial Infarction of Creatine Kinase and Lactate Dehydrogenase Isoenzymes Compared with Total Enzymes

Creatine Kinase Isoenzyme Specificity for Myocardial Damage

Jette Kraft Hilda Aastrup and P Schrøder

From the Departments of Clinical Chemistry and Internal Medicine, Sønderborg Hospital Sønderborg and the Medical Department Haderslev Hospital Haderslev Denmark

ABSTRACT The diagnostic value of creatine kinase (CK) and lactate dehydrogenase isoenzymes was investigated in a prospective study of 201 patients with suspected acute myocardial infarction (AMI) The isoenzymes were analyzed with a column chromatographic method The patients' final diagnoses were made according to the WHO criteria without knowledge of the isoenzyme results The prevalence of AMI was 50% The diagnoses were predicted with significantly greater reliability with the isoenzyme than with the total enzyme determinations in most of the patients However the greater diagnostic reliability had sufficient therapeutic consequence to justify the extra analytical cost only in patients for whom the diagnosis must be based mainly on the enzyme results The CK isoenzyme specificity for myocardial damage was studied in populations with low prevalence of heart disease In a group of 39 patients who had elevated total CK due to non cardiac disease there were five with elevated isoenzyme values, but since among 69 young healthy persons none had elevated isoenzymes this was taken to indicate that the isoenzymes may be leaked into the blood from other organs than the heart

One of the diagnostic criteria for acute myocardial infarction (AMI) is elevated values of lactate dehydrogenase (EC 1 1 2 3) (LDH) creatine kinase (EC 2 7 3 2) (CK) and aspartate aminotransferase (EC 2 6 1 1) (ASAT) in serum Since these enzymes can be elevated for several reasons interest has focussed lately on analysis of isoenzymes derived more specifically from the myocardium

The LDH isoenzymes are called LDH_{1-5} after their electrophoretic mobility In AMI the two fastest moving LDH_{1-2} are elevated The CK isoenzymes are called CK_{1-3} after their electrophoretic mobility or BB MB and MM after the composition of their subunits CK_2 or MB is reported to be specific for myocardial damage (7) if a few rare conditions such as muscular dystrophy (5) and myositis (2) can be excluded

The original electrophoretical separation of the CK isoenzymes was time consuming and not very sensitive Recently Mercer (10 11) described a column chromatographic method for separation of LDH and CK isoenzymes quickly and in one procedure The present work uses a modification of this method in a prospective study of the value of isoenzyme analysis in the diagnosis of AMI The same method was used in an evaluation of the specificity of CK_2 isoenzymes for myocardial damage

STUDY POPULATIONS

Serum samples were obtained from groups of patients with different prevalences of heart disease and from healthy persons

Group I with a high prevalence of heart disease consisted of 201 patients admitted consecutively with suspected AMI to the coronary care units of two hospitals Blood samples for isoenzyme analysis were drawn from each patient on admission and on the two following mornings at the same times as samples were drawn for the ordinarily requested total enzyme determinations Serum was separated immediately and stored at −40°C for up to two months before analysis

The isoenzyme results were considered positive if at least one of the three serum samples showed values above the discrimination value for each isoenzyme The discrimination value chosen for CK_2 was just above the

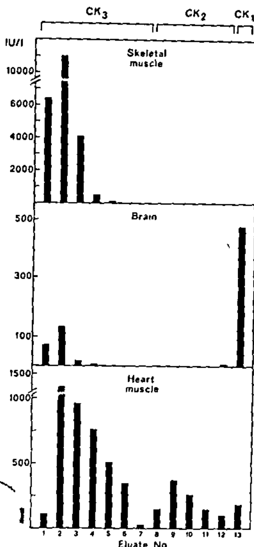


Fig 1 Chromatographic pattern of CK isoenzymes in tissue extracts

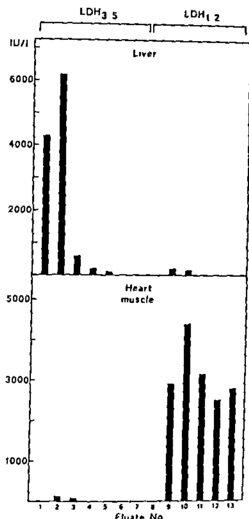


Fig 2 Chromatographic pattern of LDH isoenzymes in tissue extracts

analytical limit of detection for LDH_{1-2} it was the upper limit of reference calculated as the 97 5% percentile of the admission values from the patients who had the final diagnosis of no AMI

The patients final diagnoses were made according to the criteria set up by the WHO (20) without knowledge of the isoenzyme results Three categories of final diagnosis— definite AMI no AMI and possible AMI —are defined in detail by these criteria based on history (typical or atypical) ECG (unequivocal serial changes equivocal changes or no changes) serum total enzymes (transient equivocal or no elevations) and post mortem examination (naked eye evidence of fresh infarction or coronary occlusion)

Group 2 with a low prevalence of heart disease comprised 39 hospitalized persons in whom total CK was elevated to at least three times the upper limit of reference due to postoperative state traffic accident pulmonary embolism and in one case cerebral hemorrhage One blood sample was drawn from each patient on the morning after the episode or operation

Group 3 the control group consisted of 69 young healthy persons laboratory staff and young men in military service

METHODS

Analytical methods

Mini-columns were prepared as described by Mercer (10) DEAE Sephadex A 50 ion-exchange in Tris hydrochloride buffer (0 05 M pH 8 0) with 0 1 M sodium chloride was poured into Pasteur pipettes to make columns with the dimensions 0 5×6 cm Samples of 1 ml were applied to the columns and eluted stepwise with 6×1 ml buffer with 0 1 M sodium chloride pH 8 0 4×1 ml buffer with 0 2 M sodium chloride pH 8 0 and 2×1 ml

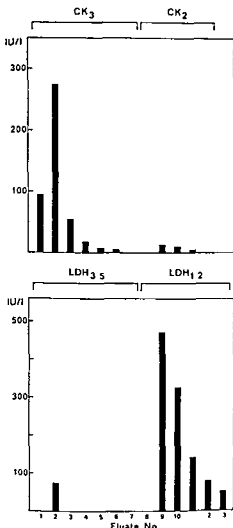


Fig. 3. Typical chromatographic isoenzyme pattern in serum from a patient with AMI less than 48 hours ago. Total CK was 470 IU/l (upper limit of reference 60) and total LDH 1 340 IU/l (upper limit of reference 450).

buffer with 0.3 M sodium chloride, pH 7.0. The effluents were collected in 13 fractions of 1 ml. The flow rate of the starting buffer was 0.3 ml/min. The CK activity in each fraction was assayed by a kinetic test at 37°C with Boehringer's Pack for Automated Analysis (sample volume 40 μl) on a LKB 8600 Reaction Rate Analyzer. The LDH activity was assayed with a Greiner Selective Analyzer (GSA II) using a modification of the Scandinavian Recommended Method with correction for blank values (8).

The isoenzyme content in each fraction was identified by chromatography of tissue extracts. Tissue samples were taken at autopsy from grossly normal human heart, brain and liver and homogenized in the Tris hydrochloride buffer with 0.1 M sodium chloride. Skeletal muscle CK was obtained as lyophilized extract of rabbit muscle CK which has the same isoenzyme composition as CK in human skeletal muscle (3) and reconstituted with the same buffer. The CK isoenzyme patterns are shown in Fig. 1. Skeletal muscle CK consists of CK_3, which is recovered in eluates 1–7. Brain CK consists of CK_3 (eluates 1–7) and CK_1, recovered in eluate 13. Heart muscle CK consists of CK_3 (eluates 1–7) and CK_2 recovered in eluates 8–12. In high activity samples of skeletal muscle CK, trace amounts of CK_2 were found (too low to be visible in the figure). In heart muscle extract a low peak of CK_1 is seen in eluate 13. This was not found in our pilot experiments and its presence may be due to dehybridization of the dimer CK_2 (M and B subunits) to CK_3 (M subunits) and CK_1 (B subunits) caused by storage for 5 months. No changes were seen in a serum pool stored for two months at −40°C. CK_1 was never detected in serum samples.

The LDH isoenzymes were separated in the same procedure but into only two fractions (Fig. 2). LDH_{3-5}, which dominate in liver together with CK_3, and LDH_{1-2}, which dominate in heart muscle together with CK_2. In serum samples (Fig. 3) both LDH_{1-2} and CK_2 had their highest concentration in eluate 9, and to avoid dilution by mixing high and low concentrations we decided to use the value of this eluate as an expression of the content of isoenzyme.

Complete separation of the CK_3 and CK_2 fractions could be obtained up to total CK about 500 IU/l, as demonstrated by rechromatography (upper limit of reference 60 U/l). Although the capacity for separating CK_3 and CK_2 as two peaks was never exceeded, even with total CK 8 000 IU/l, the CK_2 values became too high if total CK was above 450–500 IU/l because of CK_3 carry over, and rather imprecise. The amount of CK_3 carry over in eluate 9 was less than 80% of eluate 8 by elution with CK_3 buffer only. Higher values thus meant that CK_2 was present in the sample.

The total working time for about 20 samples was about 2½ hours, and the results could be reported within 6 hours after drawing the samples. The time and work needed for a single sample made the analysis unsuitable for emergency use.

Statistical methods

The diagnostic value of the analyses was calculated according to Bayes' theorem (21) and compared with the corresponding value of total enzyme determinations by means of χ^2 tests.

RESULTS

Diagnostic value of isoenzyme determinations

The typical chromatographic pattern of the isoenzymes in serum from a patient with AMI (Fig. 3) was identical with that found in extract of heart muscle (Figs. 1 and 2) except that the separation was better in serum samples. The changes in isoenzyme concentration with time are shown in

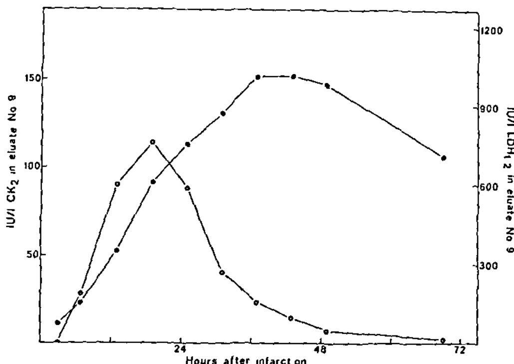


Fig 4 Typical time pattern of serum CK_2 (O—O) and $LDH_{1\ 2}$ (●—●) in a patient with AMI Samples were drawn at 6-hour intervals The discrimination value for CK_2 was 2 IU/l and for $LDH_{1\ 2}$ 130 IU/l The highest concentrations of total CK and LDH were 1400 and 2300 IU/l respectively

Fig 4 The total enzymes had maximum values in the same samples as the isoenzymes

As no differences were found in the clinical ma nal from the two hospitals the results have been ated together

Thirteen of the 201 patients in group 1 were given the final diagnosis possible AMI according to the WHO criteria Since a definitive diagnosis could not be established in these patients they were ex cluded Of the remaining 188 patients 97 had defi nite AMI and 91 no AMI (Table I) A varying number of isoenzyme results dropped out some patients lacked $LDH_{1\ 2}$ results because of instru ment breakdown and some had incomplete blood samples drawn Furthermore patients with more than 48 hours between the infarction and the first blood sample had their CK_2 results omitted be cause our data showed that CK_2 was undetectable 48 hours after the stated time of the infarction in 20% of the patients with the final diagnosis of defi nite AMI

Table I shows that the prevalence of AMI was about 50% There were 3% false positive results with CK_2 analysis alone 10% with $LDH_{1\ 2}$ analysis alone and none with combined CK_2 and $LDH_{1\ 2}$ analysis False negative results were found in 2% with CK_2 analysis alone 14% with $LDH_{1\ 2}$ anal ysis and in 2% with combined analysis

From the figures in Table I we calculated the nosographic sensitivity and specificity for AMI and

Table I *Isoenzyme results compared with final diagnosis in patients with suspected AMI*

Isoenzyme values were considered to be elevated if at least one value was above the discrimination value

	AMI	No AMI	Total
CK_2			
Elevated	85	3	88
Not elevated	2	87	89
Total	87	90	177
$LDH_{1\ 2}$			
Elevated	74	8	82
Not elevated	12	74	86
Total	86	82	168
$CK_2+LDH_{1\ 2}$			
Both elevated	68	0	68
One elevated	9	11	20
Not elevated	2	70	72
Total	79	81	160

Table II *Calculated nosographic and diagnostic value of CK and LDH isoenzymes compared with total enzymes in patients with suspected AMI*

$$\text{Sensitivity} = \frac{\text{Patients with definite AMI and positive test}}{\text{All with definite AMI}}$$

$$\text{Specificity} = \frac{\text{Patients with no AMI and negative test}}{\text{All with no AMI}}$$

$$\text{Predictive value of positive test (PV pos)} \quad \frac{\text{Patients with true positive test}}{\text{All with positive test}}$$

$$\text{Predictive value of negative test (PV neg)} \quad \frac{\text{Patients with true negative test}}{\text{All with negative test}}$$

	Isoenzymes			Total enzymes		
	CK_2	$LDH_{1,2}$	CK_2+$LDH_{1,2}$	CK	LDH	CK+LDH
Sensitivity	0.98	0.86	0.86	0.93	0.91	0.88
Specificity	0.97	0.90	0.86	0.76	0.80	0.65
PV pos	0.97	0.90	1.00	0.79	0.85	0.93
PV neg	0.98	0.86	0.97	0.92	0.88	0.91

the predictive values of positive and negative isoenzyme results (Table II) The corresponding values for the total enzymes with the upper limits of reference as discrimination values are also shown in Table II with the reservation that a full comparison is not feasible because the final diagnoses were based in part on total enzyme results Total CK results were omitted from the calculations if the infarction had occurred more than 72 hours before admission It appears from Table II that the isoenzyme values were generally higher than the values for the total enzymes The predictive value of a positive result (PV pos) for CK_2 with 3% false positive results was significantly higher than for total CK with 21% false positive results ($p<0.001$) The difference between PV pos for CK_2+$LDH_{1,2}$ and total CK+LDH was also statistically significant ($p<0.05$) while the difference between PV pos for CK_2 alone (0.97) and CK_2+LDH_{1-2} (1.00) was not As regards the predictive value of a negative result (PV neg) the difference between CK_2 and total CK had a p value of 0.09 The clinical significance of this is discussed later

Many of the patients with definite AMI had a level of total CK that was more than ten times the upper limit of reference If this value had been used as the discrimination value for total CK PV pos would have been 0.97 the same as PV pos for CK_2 If in the same patients total LDH was more than 2 1/2 times the upper limit of reference PV pos would be 1.00 This combination of very high total enzyme values which always predicted definite AMI was found in one third of the patients with AMI

About half of the patients with AMI had elevated CK_2 in the blood sample drawn on admission somewhat fewer had elevated $LDH_{1,2}$ (Fig 5) 99% of the patients had at least one positive CK_2 result and 95% one positive $LDH_{1,2}$ result in one of the first two samples

Table I shows that three patients had false positive CK_2 results In order to find out whether they

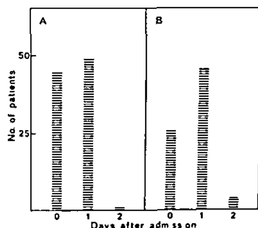


Fig 5 No of patients with first elevated isoenzyme value on the day of admission and the two following days A=CK_2 B=$LDH_{1,2}$

Table III *Number of false positive isoenzyme results in patients with other cardiac conditions than AMI*

Diagnosis	No of pats	CK_2	CK_2+ LDH_{1-2}
Atrial fibrillation*	12	0	0
Angina pectoris	14	1	0
Heart insufficiency*	15	1	0
Total	42	2	0

* Lasting for more than one day

could be of cardiac origin we examined the results for the patients with no AMI who suffered from other heart diseases (Table III) Two of the patients with false positive CK_2 results were found in this group *The other 40 patients did not have elevated* isoenzymes DC counter shock was given to three patients without causing isoenzyme elevations Two patients had been given intracardiac injection, they were found to have elevated CK_2 values but since they also had definite AMI the causal relation to the injection is unclear None of the 201 patients had ever been subjected to cardiac surgery or suffered from myocarditis or muscle diseases

Specificity of CK_2 for myocardial damage

...ong patients with high total CK not caused by ... there were 13% false positive results for CK_2 and 5% for CK_2+LDH_{1-2} (Table IV) Two of patients with elevated CK_2 had had femoral amputation but both also had cardiac disease one an affection of the mitral valves and the other atrial fibrillation and heart insufficiency Of the remaining three patients one had had the gallbladder removed and two had been operated on for intestinal tumours

Among the healthy young controls there were no false positive results (Table IV) Their total CK ranged from below the upper limit of reference values for the patients with suspected AMI up to seven times that value

DISCUSSION

Like other authors who used a column chromatographic method for CK isoenzyme separation (9 13, 22) we found it necessary to use larger eluate volumes than Mercer (10 12) to obtain complete elution This resulted in dilution of the isoenzymes and a correspondingly lower analytical sensitivity The problem could be solved in various ways One would be to concentrate serum or the eluates but this would require an extra operational procedure Another method would be to increase the sensitivity of the enzyme assay we found it possible to do this by using concentrated reagents and larger sample volumes but since this lowered all the values and reduced the range of linearity considerably we did not find the solution acceptable Our final solution was to use the peak value eluate as an expression of the isoenzyme activity With this modification the column chromatographic method had high analytical sensitivity and capacity for the detection of CK_2

In future work evaluation of infarct size may be of interest and quantitative isoenzyme results therefore necessary *By using 10 ml of CK_3 buffer instead* of 6 ml (thereby lengthening the elution time by half an hour) we found it possible to obtain correct CK_2 values i e uncontaminated with CK_3 carry-over up to total CK of about 2000 IU/l

As mentioned in the introduction elevation of serum CK_2 has been considered to be specific for myocardial damage except in some patients with muscle disease Nevertheless there were 13% false positive CK_2 results in the patient group with high total CK due to non cardiac diseases Since none of the healthy controls showing a wide range of total CK had elevated isoenzymes analytical errors are an unlikely explanation The observation may therefore rather indicate that CK_2 is not absolutely specific for myocardial tissue There is conflicting evidence for its presence in other tissues (4, 6 14 17) Some authors detected small amounts of CK_2 in tissue extracts from the gastrointestinal tract (6 17)

Our results regarding the value of isoenzyme analysis in predicting AMI agree on the whole with those of others who used various analytical

Table IV *Number of false positive isoenzyme results in populations with low prevalence of myocardial disease*

	No of persons	CK_2	CK_2+ LDH_{1-2}
Pats with non cardiac disease causing high total CK	39	5	2
Healthy young persons	69	0	0

methods (1 18 19) The isoenzymes were in most of the patients found to be both more sensitive and specific for AMI than the total enzymes The difference in PV neg between CK_2 and total CK reached statistical significance only at the 91% level It is however so important for the therapeutic consequences to have the highest possible predictive value of a negative result that we accepted this difference as being valid in order to avoid statistical type 2 error

Among the patients with AMI there were two with false negative CK_2 results An explanation of this could be that the blood samples were drawn at intervals of up to 24 hours so that small elevations of short duration may have been missed Some authors who obtained the samples every six hours found no false negative results (15) However this resulted in a considerably higher work load Other authors who found a PV neg of 1 00 for CK_2 used diagnostic criteria that were different from ours (18 19)

In addition to the greater diagnostic reliability we thought it might be possible to obtain an earlier diagnosis with isoenzyme determinations because while transient elevations and therefore at least three values are necessary for a total enzyme diagnosis one elevated isoenzyme value was sufficient for a positive isoenzyme result But although a positive diagnosis was thus obtainable in most cases within the first day after admission it would have limited therapeutic consequences and a negative diagnosis could not be made with 100% certainty

Recent work (16) has shown that adenylate kinase (EC 2 7 4 3) may in some patients cause falsely elevated CK values and conceivably also CK_2 values We have not investigated this relation

The conclusion of this study is that in spite of the greater diagnostic reliability obtainable with isoenzyme determinations both for negative results and for positive results in the two thirds of patients without very high total enzymes its therapeutic consequences will in most cases not be sufficient to justify the extra analytical cost The use of diagnostic isoenzyme analysis should therefore be restricted to cases in which the diagnosis must be based mainly on the enzyme results

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Table I *Renal findings in patients with early diastolic murmur*
SR=slightly reduced Es=end stage D=dialysis

Pat no	Age (y)	Sex	Renal disease	Renal status	BP (mmHg)	Hb (g/dl)	Serum creatinine (μmol/l)
Group I (organic heart disease)							
1	49	♂	Collagen	SR	170/90	10 9	169
2	46	♂	Amyloidosis	SR	210/80	8 6	230
3	30	♀	Chronic glomerulonephritis	Es D	110/40	5 9	–
4	60	♂	Chronic glomerulonephritis	Es D	170/100	6 5	1 264
5	60	♂	Paramyloid	Es D	140/70	5 4	–
6	47	♀	Chronic glomerulonephritis	Es D	170/90	6 2	1 679
7	47	♂	Chronic glomerulonephritis	Es D	240/–	4 6	795
8	61	♀	Renal tuberculosis	Es D	160/80	9 8	663
Group II (no heart disease positive angiography)							
9	18	♀	Chronic pyelonephritis	Es D	150/100	9 2	654
10	55	♂	Nephrosis	Es –	180/90	5	521
11	58	♀	Chronic glomerulonephritis	Es –	260/115	10	495
12	30	♂	Diabetic nephropathy	Es	180/95	11	336
Group III (no heart disease negative or no angiography)							
13	53	♂	Chronic glomerulonephritis	Es D	180/100	5 8	857
14	37	♂	Renal tuberculosis	Es D	240/115	6 0	1 361
15	55	♂	Renal tuberculosis	Es D	220/115	4 8	–
16	46	♂	Interstitial nephritis	Es D	180/110	4 0	1 061
17	57	♂	Chronic glomerulonephritis	Es D	150/100	4 0	822
18	26	♂	Chronic glomerulonephritis	Es D	160/105	5 3	–
19	31	♂	Amyloidosis	Es D	130/80	9 3	844
20	58	♂	Renal tuberculosis	Es D	150/70	7 0	–
21	58	♀	Nephrosclerosis	Es D	215/110	7 3	660

murmur was heard along the left sternal border Three of these patients have been treated with hemodialysis and the diastolic murmur has disappeared during treatment

Patient 9 This 18 year-old girl was admitted to the Medical Department in 1973 with a history of renal failure of 3 months duration On admission her BP was 180/115 There was no edema No cardiac enlargement A diastolic decrescendo murmur was heard along the left sternal border with maximum intensity in the third left interspace Serum creatinine was 654 μmol/l Hb 9 2 g/dl Angiocardiography at that time showed aortic insufficiency of grade 2–3 normal coronary arteries normal pressure in the left ventricle Hemodialysis was started in 1974 and in June 1974 renal transplantation was carried out The diastolic murmur had then disappeared Renal transplantation was followed by repeated rejections Hemodialysis was therefore started again She developed sinoatrial block which had to be treated with pacemaker In Sept 1974 she collapsed and died suddenly Autopsy disclosed an enlargement of the left ventricle The heart weighed 410 g There was no dilatation of the aortic ostium which measured 5 7 cm There was slight fibrosis of the central nodulus in the aortic valves which were otherwise normal In the left atrial wall several calcified foci were found

Patient 10 This 55-year-old man had proteinuria since 1944 and hypertension since 1971 and progressing renal failure with a serum creatinine of 521 μmol/l On admission in June 1973 his BP was 185/110 There was a diastolic decrescendo murmur at the left sternal border Angiography showed aortic insufficiency of grade 2 There was some enlargement of the left ventricle with normal pressures Selective cineangiography of the coronary arteries was normal Renal transplantation was carried out in Nov 1973 Since then his renal function has been normal BP is normal The diastolic murmur has disappeared

Patient 11 This 49-year-old woman has had proteinuria for 20 years and hypertension for 12 years She was treated for cancer ovarii with cobalt irradiation in 1964 In Jan 1976 she had malignant hypertension BP 270/140 serum creatinine 495 μmol/l On admission in April 1975 she had papilledema She complained of angina pectoris There was a diastolic decrescendo murmur along the left sternal border Angiocardiography showed slight dilatation of the left ventricle with an increased pressure of 270/10–25 There was a slight aortic insufficiency of grade 1 The coronary arteries were normal On treatment with antihypertensives digitalis and saluretics her condition has improved In Aug 1976 her BP was 150 80 serum

creatinine 425 μmol/l Hb 12 6 g/dl The diastolic murmur has disappeared

Patient 12 This 30-year-old man had had diabetes from the age of 11 years and had been treated with insulin and diet In 1971 he had myocardial infarction with cerebral embolus In 1974 diabetic retinopathy and nephropathy with a creatinine clearance of 16 ml/min were found In 1975 treatment was started for hypertension His BP was then 240/110 Serum creatinine 336 μmol/l On admission in June 1966 his BP was 150/95 There was no cardiac enlargement A diastolic murmur was heard along the left sternal border Angiography showed an elevated end diastolic pressure in the left ventricle of 16 mmHg and enlargement of the left ventricle with dyskinesia of the posterior wall Aortography showed aortic insufficiency of grade 1 Selective cineangiography showed significant stenosis of the right coronary artery As a pretreatment for renal transplantation proximal gastric vagotomy was carried out in Oct 1976 This was followed by myocardial infarction pulmonary edema and repeated embolization to the femoral arteries where embolectomy had to be carried out twice He died on Oct 10th 1976 Autopsy showed old and recent myocardial infarction cerebral embolization and diabetic nephropathy The aortic valves were normal and there was no dilatation of the aortic ostium (circumference 7 0 cm)

Group III (patients with negative or no angiocardiography)

Group III comprises 9 patients with no proven aortic insufficiency Angiocardiography was not carried out in 7 of them In the remaining 2 patients angiocardiography failed to demonstrate aortic insufficiency at a time when the diastolic murmur was still audible

Patient 14 This 37 year-old man had renal tuberculosis in 1942 Right sided nephrectomy was carried out in 1952 In the following year one half of the left kidney was resected and the patient received treatment with tuberculostatics Since then no tubercle bacilli have been found in his urine Hypertension since 1969 In April 1973 he developed severe renal failure He was admitted to the Medical Department in June 1973 with a BP of 220/120 and enlarged heart and pulmonary edema Serum creatinine was 857 μmol/l A diastolic murmur was heard along the left sternal border On angiocardiography the left ventricle was normal There was no aortic insufficiency There was a subtotal occlusion of the right coronary artery The heart was enlarged The diastolic pressure in the left ventricle was normal He was treated with hemodialysis and the diastolic murmur disappeared Renal transplantation was carried out in March 1974 He now has slightly reduced renal function serum creatinine 141 μmol/l BP 160/90

Patient 21 This 58-year old woman had pulmonary tuberculosis in 1956 For 1 year she has experienced increasing dyspnea and angina pectoris anorexia vomiting and weight loss Serum creatinine increased to 640 μmol/l BP rose to 250/130 On admission to the Medical Department in Nov 1976 her BP was 210/110 The heart was enlarged to the left There was a holodiastolic murmur along the left sternal border On angiocardiography there was no aortic insufficiency but a significant stenosis on the main left coronary artery The left ventricle was normal and diastolic pressure there was 24 mmHg She has been treated with hemodialysis Her diastolic murmur is still present as a high frequency murmur midway between apex and the left sternal border The diastolic murmur is holodiastolic with maximum intensity after the second heart sound (Fig 1)

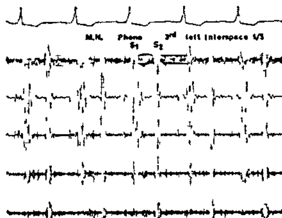


Fig 1 Phonocardiogram of patient 21

DISCUSSION

The diastolic murmur found in patients with severe renal insufficiency has been attributed to functional aortic insufficiency and has been variously ascribed to hypertension fluid overload or anemia The murmur has in some cases been heard at or near the apex (11) In our patients the murmur was heard at the left sternal border with its maximum in the third or fourth left interspace A diastolic murmur of this character and location might be due to pulmonary or aortic valve insufficiency Pulmonary insufficiency cannot be thoroughly excluded as no pulmonary angiography has been carried out in these patients There was however no indication of pulmonary hypertension either by auscultation by X ray of the heart or by ECG The character and localization of the murmur would indicate aortic insufficiency

Among the four patients in group II in whom aortic insufficiency has been demonstrated by angiography BP varied from 150/100 to 260/115 Severe hypertension thus does not seem to be a pre

Table II *Cardiac and angiographic findings in patients with early diastolic murmur*
LSB=left sternal border LVS=left ventricular strain AI=aortic insufficiency MI=myocardial infarction LVEDP=left ventricular end-diastolic pressure AS=aortic stenosis

Pat no	Previous illness	Diastolic murmur	Congestive heart failure	Heart volume (ml/m²)	ECG	Angiography
Group I (organic heart disease)						
1	Rheumatic fever	LSB	Yes	640	LVS	AI MI LVEDP 40 mmHg
2	Chronic polyarthritis	LSB	Yes	520	LVS	AI coronary stenosis LVEDP 30 mmHg
3	Previous endocarditis	LSB	Yes	765	LVS	AI MI LVEDP 33 mmHg
4	–	LSB	Yes	Enlarged	LVS	Coronary stenosis AS
5	–	LSB	Yes	1 260	Bifascic block pacemaker	–
6	Scarlatina	LSB	Yes	670	Junctional bradycardia pacemaker	AI
7	–	LSB	Yes	505	LVS	–
8	–	3rd left interspace	No	530	LVS	Patent ductus arteriosus
Group II (no heart disease positive angiography)						
9	–	LSB	No	235	LVS	AI
10	Rheumatic fever	LSB	Yes	550	AV block I	AI
11	–	LSB	Yes	450	Left axis deviation	AI LVEDP 10 mmHg
12	–	LSB	No	470	LVS	AI coronary stenosis LVEDP 16 mmHg
Group III (no heart disease negative or no angiography)						
13	Scarlatina	LSB	Yes	765	LVS	No
[illegible]	–	LSB	Yes	610	LVS	No AI following dialysis stenosis of right coronary artery
15	–	LSB	No	?	Left axis deviation	No
16	Scarlatina	LSB	No	790	LVS	No
17	–	LSB	Yes	700	LVS	No
18	–	LSB	No	Enlarged	LVS	No
19	Bechterew	LSB	Yes	Enlarged	Left axis deviation	No
20	–	LSB	No	–	LVS	No
21	Pulmonary tuberculosis	LSB	Yes	Enlarged	LVS	Stenosis of left coronary artery

requisite for the murmur All these four patients were anemic Functional diastolic murmurs have been described in patients with anemia but without organic heart disease Thus Goldstein and Boas (7) heard diastolic murmurs in 6 of 39 patients with pernicious anemia In our patient 13 in group III the diastolic murmur was heard with a Hb of 15 4 g/dl Anemia thus does not seem to be a prerequisite for the murmur The appearance of the murmur in patients with advanced congestive heart failure and other signs of fluid overload and its disappearance on treatment with hemodialysis suggest that fluid overload may be the most important pathogenetic mechanism

Autopsy has been carried out in only two of the patients with supposedly functional aortic insufficiency (nos 9 and 12) Neither of these patients presented pathological changes in the aortic valves or in the aortic valve ring which might indicate an organic origin of the murmur

Recently Barratt et al (3) have challenged the view that aortic insufficiency is the cause of this

)ied	Autopsy
	No
following renal trans plantation	Calcified aortic valves
	Paramyloid kidney heart
following renal trans plantation	Endocarditis myocarditis Pneumonia pulmonary embolism
following renal trans plantation	Myocardial calcification
†	MI
†	No
†	No
†	No
†	No

diastolic murmur They have done angiocardiography in 6 patients with a diastolic murmur and detected aortic regurgitation in only one All their patients were in end stage renal failure and most of them had fluid overload The BP was normal in 2 of their patients and elevated in 4 Severe hypertension was found only in 2 patients Hb ranged from 6 0 to 10 6 g/dl Two of their patients died and autopsy showed no pathologic changes in the cardiac valves They suggested that the murmur might have been caused by pericarditis as they detected a small amount of pericardial effusion by echocardiography

Our findings are at variance with the above study In our patients there was no indication of pericarditis Pericarditis is frequently seen in end stage renal failure but the murmur has quite different characteristics like a systolic-diastolic to-and fro rub The appearance of the diastolic murmur during periods of advanced failure and its disappearance following treatment of renal failure by hemodialysis coupled with the character of the murmur suggest that it is due to functional aortic insufficiency Furthermore aortic insufficiency was demonstrated by angiocardiography in 4 of the 6 patients in whom it was carried out

Diastolic murmurs have been observed in patients with coronary artery stenosis Lund Larsen (8) reported on a patient with a holodiastolic murmur in the fourth intercostal space at the left sternal border Angiocardiography showed severe stenosis of the left coronary artery This patient later had myocardial infarction whereupon the diastolic murmur disappeared Dock and Zoneraich (5) similarly observed a diastolic murmur in a patient with severe stenosis of the left anterior descending coronary artery It is thus possible that the diastolic murmur heard in our patient 21 may be caused by coronary artery stenosis She had a severe stenosis of the main left coronary artery and the murmur still persists although her general condition has improved

Patient 14 also had coronary artery stenosis but in this case of the right coronary artery Here the murmur disappeared following hemodialysis It is therefore improbable that it was caused by coronary artery stenosis As far as we know diastolic murmur has not been observed in patients with stenosis of the right coronary artery

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Listeria Encephalitis in Five Renal Transplant Recipients

Per Magnus Niklasson Anna Hambraeus Goran Lundgren Gosta Magnusson Par Sundelin and Carl Gustav Groth

From the Department of Infectious Diseases Roslagstull Hospital the Division of Transplantation Surgery the Department of Clinical Microbiology and the Department of Pathology Huddinge Hospital Karolinska Institute Stockholm Sweden

ABSTRACT Listeria encephalitis has occurred recently in 5 renal transplant recipients at the Transplantation Unit in Stockholm Symptoms from the central nervous system, such as coma hemiparesis and cranial nerve paresis dominated the clinical picture Listeria monocytogenes was isolated from the blood of all the patients, from the cerebrospinal fluid in two from the urine in one and post mortem from the brain in one patient Pleocytosis never exceeded 200 leucocytes/mm^3 and the glucose ratio was normal or near normal Complement fixation test for Listeria was negative in all 5 patients Four patients expired in spite of treatment with large doses of penicillin and other antibiotics to which the isolated strains were sensitive in vitro At autopsy, inflammation and necrosis were observed in the brain, especially the brain stem, and there was mild lymphocytic infiltration of the meninges The surviving patient was treated with a combination of chloramphenicol and ampicillin

Infection by Listeria monocytogenes afflicts mainly the elderly the very young and patients suffering from debilitating and especially malignant diseases Listeriosis has moreover been found to be more common in patients receiving immunosuppressive therapy (4) and listerial infection has been reported in renal transplant recipients (1 4 8–11)

We report 5 cases of listeriosis that have occurred recently in renal transplant recipients at the Transplantation Unit in Stockholm All the patients had the malignant encephalitic form of the disease In the 4 patients who succumbed extensive necrotic inflammatory changes were found in the brain especially the brain stem

Address reprint requests to Dr C G Groth Huddinge Hospital S 141 86 Huddinge Sweden

PATIENTS AND METHODS

Between 1964 and 1976 a total of 337 patients underwent renal transplantation in Stockholm The first 4 cases of listerial infection reported here occurred over a period of 11 months (Aug 1974–July 1975) the fifth case was diagnosed in June 1976 These are the only known cases of listeriosis in the Stockholm series

Three of the patients received a cadaveric graft and 2 a maternal kidney The kidneys were revascularized to the iliac vessels and located extraperitoneally using standard techniques Immunosuppressive therapy comprised azathioprine and prednisone in standard doses Three of the patients (nos 2 4 and 5) were also given antilymphocyte globulin (Behring Werke) 15 mg/kg daily for 9–21 days If a rejection episode occurred 1 g of α methylprednisolone was injected i v on three consecutive days the oral dose of prednisone was increased to 150–200 mg daily and then gradually lowered to a maintenance level

Triplicate samples of venous blood were used for blood culturing Cultivation was performed aerobically and anaerobically (95% N_2 and 5% CO_2) in closed bottles containing brain heart infusion (Difco) agar slants with para aminobenzoic acid and meat infusion broth containing penicillinase About 0 5 ml of blood was inoculated in each bottle at the bedside In the event of suspected growth subcultures were performed on blood agar and haematin agar Urine was cultured on blood agar and CLED medium (Oxid) Gram stained cerebrospinal fluid (CSF) smears were examined immediately Samples were then cultured on blood and haematin agar plates and in the liquid media used for the blood samples

Samples taken at autopsy were plated on blood agar for aerobic and anaerobic incubation and on haematin agar and CLED medium for aerobic incubation only The material was then inoculated in enrichment broth

Table 1 *Summary of clinical and laboratory findings in five renal transplant recipients with Listeria encephalitis*

Case no	Onset of infection (mo after transplantation)	Survival after onset of infection (d)	Type of Listeria in cultures	Maximum pleocytosis (polynuclear mononuclear cells/mm³)	Maximum protein content in CSF[a] (g/l)	Glucose (mmol/l)		
						In CSF	In blood[b]	Ratio
1	6	17	Blood 1 CSF 1	100–100	0 99	2 5	3 9	0 6
2	1	194	Urine 1 Blood 1 4 Brain tissue 1 4	–	–		–	
3	5	114	Blood 1 4	104–28	1 03	2 3	4 3	0 5
4	4	51	Blood 4 CSF 4	0–15	1 57	5 3	8 9	0 6
5	1	Alive at 9 mo	Blood 1 4	None	0 83	5 7	9 5	0 6

[a] Normal 0 15–0 50 [b] Normal 3 1–5 6 [c] Normal ≥0 6

L monocytogenes was identified on the basis of morphology and biochemical reactions Serological typing was performed with commercially available sera (Difco) *Complement fixation test for L monocytogenes was performed on patient serum specimens*

CASE REPORTS

Case 1

A 28-year-old woman with chronic pyelonephritis and mia who had been treated with dialysis for 2 years ved a cadaveric kidney transplant (3 HL-A donor–nt incompatibilities) in Feb 1974 Transplant func as good initially and there were no rejection epi es

In Aug 1974 the patient suddenly developed fever (39 5 C) and an erysipelas like erythema on her left leg A culture from the skin was negative A gram positive rod that grew in one of three blood cultures after 4 days of incubation was regarded as probably a contaminent The peripheral blood leucocyte count was 6700/mm³ with slight neutrophilia Initially penicillin was given orally but when Staphylococcus albus grew in two of nine blood cultures treatment was changed to i v cephalothin As there was no clinical improvement a change was made to cloxacillin and gentamycin The patient became afebrile but suddenly developed coma and cervical rigidity Examination of the spinal fluid revealed moderate pleocytosis the protein content was elevated the glucose ratio was normal (Table I) L monocytogenes grew on culture No virus could be isolated *from CSF Serological tests for* CNS viruses were negative At this time 7 days after admission it became clear that the gram-positive rod isolated initially from the blood was in fact L monocytogenes Oxytetracycline was given in daily i v doses of *2 g* with *the addition of 20 MU of penicillin but the latter* drug was withdrawn after 4 days The azathioprine was discontinued on the 9th day after admission while the prednisone therapy was continued at a low dosage (10 mg/day) The patient s condition improved slightly during the next few days but she then went into a deep coma and death ensued 17 days after the first signs of illness

Autopsy disclosed a moderately severe internal hydrocephalus The brain was oedematous and multiple haemorrhagic foci were found in the subcortical area of the *hemispheres and around the third ventricle Under the* microscope diffuse lymphocytic infiltration of the meninges was seen The brain tissue displayed multiple foci of degeneration surrounded by gliosis There was dense lymphocytic infiltration of the perivascular tissue The *kidney transplant displayed evidence of severe acute and* chronic rejection Cultures yielded no growth

Case 2

A 58 year-old man with primary hyperparathyroidism and nephrocalcinosis had been maintained on dialysis from 1971 to 1973 In April 1973 he received a cadaveric kidney (4 HL-A donor–recipient incompatibilities) Graft function was excellent initially but 4 months later chronic rejection had led to renal failure and dialysis was resumed In Jan 1974 retransplantation was performed with a cadaveric kidney (4 HL A donor-recipient incompatibilities) Six weeks later there was an acute reversible rejection episode

In Feb 1975 chronic rejection prompted a temporary increase in the prednisone dosage L monocytogenes type 4 was isolated from the urine in two routine *cultures* There were no urinary tract symptoms Ampicillin (2 g daily) was given orally for 7 weeks The urine cultures became negative and remained so

In Sept 1975 the patient was admitted to hospital with *high fever (40°C) and confusion His general condition* was poor and there was pronounced generalized oedema There was no cervical rigidity and no focal neurological signs An attempt at spinal tap failed Since septicaemia was suspected *treatment* was started immediately with penicillin i v and gentamycin The patient had recurrent generalized convulsions and died on the day after admission Three blood cultures obtained on admission grew L monocytogenes

At autopsy gross examination disclosed normal brain and meninges Microscopic examination revealed moderate focal lymphocytic infiltration of the meninges but normal brain tissue The transplanted kidney displayed evidence of advanced chronic rejection and the interstitial renal tissue contained multiple small abscesses Brain tissue culture yielded L monocytogenes Renal tissue culture grew Proteus morganii

Case 3

A 52 year old man with polycystic kidney disease and uraemia had undergone dialysis for 4 years prior to a cadaveric kidney transplantation (3 HL A donor–recipient incompatibilities) in Nov 1974 There was never any evidence of graft rejection

In April 1975 the patient became acutely ill with headache and fever (39 5 C) Five days later he developed unilateral ptosis owing to partial paresis of the right oculomotor nerve No other neurological signs were noted

The peripheral blood leukocyte count was 9 500/mm^3 with neutrophilia The leukocyte count in CSF was 52/mm^3 The protein content was elevated the glucose ratio was slightly subnormal (Table I) CSF and blood cultures were negative No virus was isolated from the CSF Serological tests for CNS viruses were negative The patient was given gentamycin and large i v doses of penicillin for 6 and 10 days respectively The fever was depressed and there was some improvement of the ptosis

Five days after withdrawal of penicillin there was a relapse with fever and ptosis The leukocyte count in CSF was now 132/mm^3 with a majority of polynuclear cells The EEG was normal A brain radioisotope scan showed an increased uptake of the isotope corresponding to the brain convexity Cultures for bacteria virus isolation and a serological test for viruses were again negative Ampicillin (8 g daily) was given i v for 23 days after which the patient was discharged with slight ptosis as a residual symptom

On Aug 11 about 3 months later the patient became febrile (40 0°C) for the third time He also complained of headache and dizziness Two days later left sided hemiparesis appeared EEG showed right sided abnormalities in the temporal and parietal regions Two brain radioisotope scans showed the same abnormality as before Carotid angiography indicated increased intracranial pressure CSF contained only 4 leukocytes/mm^3 and 0 09 g of protein/l Two CSF cultures were negative L monocytogenes was however isolated in 5 consecutive blood cultures Penicillin (20 MU daily) was given i v The azathioprine dose was reduced from 150 to 50 mg/day the prednisone dose was reduced to 10 mg/day Following a week of slight improvement coma and respiratory arrest suddenly developed CSF was bloody Carotid angiography disclosed absence of cerebral circulation Treatment was discontinued and the patient expired

Autopsy revealed intracerebral haemorrhage which had destroyed the right internal capsule basal ganglia and upper thalamus The ventricular system was filled with blood The rostral part of the pons contained small haemorrhages The meninges showed focal lymphocyte infiltration The haemorrhages were ascribed to necrotizing encephalitis Cultures of liver heart kidney and lung showed no growth No brain tissue cultures were performed

Case 4

The patient was a 38 year old man suffering from juvenile diabetes mellitus with severe nephropathy In Feb 1975 after 3 months of dialysis he received a maternal renal transplant Renal function normalized initially but after 6 weeks there was an acute reversible rejection episode

In July the patient suddenly developed transient numbness in all extremities and two days later right sided hemiparesis There were no signs of infection A spinal tap revealed no pleocytosis but the protein content was elevated (Table I) CSF culture was negative Echoencephalograms were normal but an EEG disclosed a moderate left sided abnormality

Four days after the onset of hemiparesis the patient became comatose and febrile (40 8°C) The peripheral blood leukocyte count was 11 300/mm^3 with a shift to the left A brain radioisotope scan and left sided carotid angiography disclosed no anomalies L monocytogenes was now isolated from CSF and from one of 3 blood cultures Initial antibiotic treatment with penicillin (20 MU daily) was followed by rapid abatement of the fever Immunosuppressive therapy was discontinued After 10 days on penicillin the patient was still comatose and this drug was replaced by oxytetracycline (1 000 mg daily) CSF examinations revealed a maximum of 15 leukocytes/mm^3 The protein content was elevated the glucose ratio was normal (Table I) Six blood cultures and two CSF cultures during therapy were negative No virus could be isolated from the CSF and serological tests for CNS viruses were negative The patient died 7 weeks after the onset of his illness without having regained consciousness

Several brownish grey foci measuring up to 1 cm across were found in the left parietal lobe of the brain the left putamen and the brain stem Under the microscope the foci were seen to consist of a central necrotic area surrounded by tissue displaying gliosis and perivascular lymphocytic infiltration

Multiple microabscesses were found in the liver heart and transplant The kidney showed signs of advanced chronic rejection Cultures on autopsy specimens from blood the liver spleen and transplant yielded growth of Candida Cultures of CSF were negative Brain tissue was not cultured

Case 5

A 47 year old man suffering from nephritis probably associated with phenacetin abuse had been on a low protein diet for uraemia from 1974 to 1976 In April 1976 a maternal kidney was transplanted Renal function was restored slowly and incompletely Twenty three days after transplantation there was an acute rejection episode with renal failure and temporary haemodialysis was required The rejection was reversed with high doses of steroids

In June the patient suddenly developed fever (39 3 C) headache and diplopia, followed by right sided hemiparesis on the next day A spinal tap revealed an elevated pressure (25 cmH$_2$O) CSF contained no

content was increased but the glucose level was normal (Table I) The peripheral blood leukocyte count was 5700/mm³ A blood culture grew L monocytogenes CSF culture was negative No virus could be isolated from CSF and serological tests for CNS viruses were also negative An EEG revealed moderate functional abnormalities situated in the upper part of the brain stem A brain radioisotope scan showed an increased isotope uptake in the left parietal region At the onset of symptoms i v benzylpenicillin and trimethoprim sulfamethoxazole were administered but two days later when L monocytogenes had been found in the blood these drugs were replaced by chloramphenicol (2–4 g daily for 9 days) and ampicillin (12 g daily for 5 days and then 6 g daily for 24 days) The chloramphenicol levels determined one hour after an i v injection of 1 g were 43 and 25 μg/ml in serum and CSF respectively The corresponding ampicillin levels were 320 and 14 μg/ml The prednisone dose was reduced to a low level (10 mg/day) and azathioprine was withdrawn

Eight days after introduction of the chloramphenicol and ampicillin therapy the patient became afebrile The diplopia disappeared and the hemiparesis improved On repeated examination of the CSF the findings were unchanged normal cell counts and glucose ratio but elevated protein content EEG and brain radioisotope scan were normal Antibiotic therapy was continued for 31 days Adequate immunosuppressive steroid treatment (30 mg prednisone) and azathioprine were resumed 35 and 14 days respectively after the debut of the infection Renal function remained stable throughout Nine months after the onset of his illness the patient was in good condition with slight persistent right sided hemiparesis The serum creatinine was moderately elevated

CHARACTERISTICS OF L MONOCYTOGENES

e microorganism was of type 1 4 in two of the patients type 1 in one patient and type 4 in another In one patient L monocytogenes type 1 was detected in the urine and later on type 1 4 occurred in the blood and the brain tissue (Table I)

In vitro all the strains were highly sensitive to penicillin in minimal inhibitory concentrations (MIC) of 0 125 μg/ml or less They were also sensitive to ampicillin (MIC 0 25–0 4 μg/ml) tetracycline (MIC 1–2 μg/ml) and several other antibiotics Only in the last case was chloramphenicol included in the test the microorganisms were sensitive (MIC 2 μg/ml)

In 6 sera collected a few days to 3 months after onset of infection from the 5 patients the complement fixation test for L monocytogenes was negative

DISCUSSION

The clinical manifestations of listeriosis of the CNS include purulent meningitis meningoencephalitis and encephalitis Purulent meningitis which is the most common form of the disease cannot be differentiated clinically from other types of bacterial infections of the meninges, whereas the meningoencephalitic form is easily mistaken for viral or tuberculous infection Listeria encephalitis with a clinical picture similar to that of viral encephalitis occurs in animals but is rare in man Several cases were observed during an epidemic in Germany in 1956 Since then only sporadic cases of malignant neurolisteriosis have been reported (2 8 11) 2 of them in renal transplant recipients (8 11)

The 5 cases presented here should be classified as Listeria encephalitis In 3 patients post mortem examination revealed extensive necrotic inflammatory changes in the brain especially the brain stem similar to those described by Eck (3) In one patient no morphological abnormalities were observed in the CNS apart from slight inflammation in the meninges but at autopsy Listeria was cultivated from the brain parenchyma The rapid course of the disease in this patient with sudden death following generalized convulsions also points to a major encephalitic component The early and prominent focal neurological signs in 3 of the patients including paresis of the eye muscle and hemiparesis also indicate primary involvement of the brain Furthermore 2 patients were comatose for about 2 and 7 weeks prior to death

The only consistent abnormality disclosed by examination of the CSF was a moderate elevation of the protein content Pleocytosis was absent or moderate and mononuclear cells dominated in one case only The ratio of the glucose contents in CSF and blood was subnormal only in one patient and the CSF glucose was never below 2 3 mmol/l This is remarkable in view of the findings of Lavetter et al (6) In their series of meningeal listeriosis the prognosis seems to have been best when the initial CSF glucose level was above 1 7 mmol/l In none of our patients could the microorganisms be seen in gram stained smears of spinal fluid and in only 2 patients did cultures grow Listeria Thus the findings in the CSF were non-characteristic and not distinguishable from those in viral meningoencephalitis Blood cultures on the other hand revealed the aetiologic agent in all 5 cases The complement fixation test for Listeria antigen which was performed in all 5 patients was negative Nor has this test been found to be of much help in the diagnosis of Listeria infection in patients not receiving immunosuppressive therapy (12)

The occurrence of 5 cases of listeriosis in the same hospital ward is remarkable and suggests an

epidemiological factor However only 2 patients (nos 3 and 4) were in the ward at the same time Another curious feature is that serotyping of the isolated strains suggested different types they may still have been of the same type however as rou tine serotyping of Listeria is an insensitive method (12) Unfortunately the strains were not kept and simultaneous testing of identity was therefore im possible

In 3 patients the dosage of the immunosuppres sive drugs was greatly reduced and in another the drugs were withdrawn These measures were how-ever without any appreciable effect on the course of the disease in 3 of these 4 patients by this time it may have been too late for any recovery of the immune defence

The isolated Listeria strains were sensitive to penicillin ampicillin tetracycline and several other antibiotics All 3 of these drugs were used in our treatment schedules A bactericidal effect was ap-parently obtained also in vivo Thus no L mono-cytogenes was isolated from blood or internal or gans at autopsy except in case 2 who had been on antibiotic therapy for less than 24 hours Moreover repeated blood and CSF cultures during therapy were negative The fatal outcomes might then have been due to irreversible damage to the CNS early in the course of the disease

From experience of natural and experimental in fection with Listeria in animals it is evident that an tibiotic therapy must be introduced at a very early stage if it is to save the life of the animal with encephalitis On the other hand the possibility exists that despite the antibiotic treatment some microorganisms may persist in the brain Because of the intracellular location of Listeria behind the blood–brain barrier the bacteria are protected from the action of most antibiotics In addition the im munosuppressive therapy weakens the host s cellu lar immune response which is a critical factor in the resistance to listerial infection (7) It is evident from the second and third attacks of neurolisteriosis in our patient 3 and from the listerial infection of the urinary tract preceding the meningoencephalitis in patient 2 that Listeria may long remain dormant in the body in spite of protracted treatment with ampicillin The only surviving patient received chloramphenicol in addition to ampicillin It is pos sible that the recovery is to be attributed to the former drug which is known to reach a high con centration in both brain tissue and CSF (5)

The presence of focal neurological symptoms and signs in a renal transplant recipient should arouse suspicion of Listeria encephalitis Conven tional penicillin and tetracycline therapy seems to be ineffective in such cases The concurrent use of other antibiotics such as chloramphenicol should therefore be tried

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The Excretional Patterns of Lactate, Pyruvate and α-Ketoglutarate in Renal Transplants

Hans Erik Hansen and Poul Erik Skov

From Medical Department C Århus Kommunehospital and University of Århus Århus Denmark

ABSTRACT The excretional patterns of lactate pyruvate and α ketoglutarate were investigated after renal transplantation in 36 patients Fourteen patients had received a living donor kidney with short ischemia time and good initial graft function, 22 had a cadaver transplant with an initial ¹²⁵iothalamate clearance of more than 6 ml/min The excretion of lactate and pyruvate did not vary significantly from that seen in normal controls or patients with uremia In six patients with cadaver transplants clearance values of α ketoglutarate exceeded that of the glomerular filtration rate indicating a net tubular secretion of this substance During acute rejection episodes in 5 patients, no changes were seen in the excretional patterns of lactate pyruvate and α ketoglutarate

Investigations of the excretional patterns of lactate pyruvate and α ketoglutarate were performed after renal transplantation in order to evaluate any changes in the renal elimination of these substances after the transplantation Such changes might reflect an altered oxidative metabolism in the transplant during the time when it had been stored cool without circulation and also following relative ischemia during rejection episodes

Investigations were carried out immediately after transplantation during the early posttransplant period and at regular intervals during the first post transplant year

STUDY POPULATION

The study comprised 36 patients Blood sugar values and respiration were normal at all investigations and there were no signs of circulatory insufficiency Immunosuppression consisted of azathioprine and prednisone Azathioprine treatment was initiated in living-donor transplants 3 days before transplantation in a dose of 3 mg/kg/day Cadaver recipients were given 5 mg/kg just before transplantation After transplantation a dose of 1–3 mg/kg/day was given depending on the WBC Prednisone therapy was initiated in a dose of 150–250 mg/day 3 days after transplantation During the following year the daily dose was reduced to 10–20 mg Initial hematocrit values varied between 20 and 30% The values normalized during the following year

The patients were divided into two groups *Group I* consisted of 14 patients 6 women and 8 men who had received a living-donor kidney that is a kidney from either a parent or a sibling Their age ranged from 14 to 53 years (mean 32) In all patients total ischemia times were short range 41–67 min average 53 min Initial graft function was good ¹²⁵iothalamate clearance averaging 38 ml/min (range 26–59) During the period of investigation three patients lost their grafts due to acute rejection episodes and 1 month after transplantation 11 patients were left *Group II* comprised 22 patients 10 women and 12 men in the age range 22–59 years (mean 45) who had received a cadaveric kidney transplant These patients were selected from a total of 106 who received transplants during the period of investigation The criterion for investigation was good initial graft function with ¹²⁵iothalamate clearance of more than 6 ml/min immediately after transplantation (average 23 5 range 6–59) Total ischemia times ranged from 120 to 792 min (average 380) Perfusion with Perfudex® was employed in most cases Mechanical perfusion was not used Severe acute rejection episodes occurred in 5 patients Five patients died during the period of study and one was excluded because of ureteral necrosis leading to a permanent cutaneous ureterostomy From one month after transplantation 16 patients were left

Renal function stabilized after one month in both groups and was almost constant during the following year ¹²⁵iothalamate clarance averaging 54 and 60 ml/min respectively in groups I and II 12–14 months after transplantation (2)

METHODS

The glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) were determined in all patients

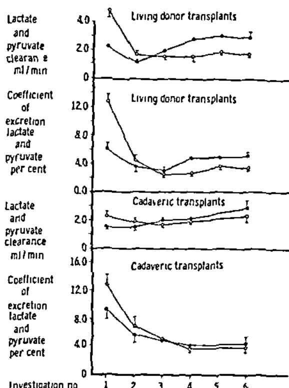


Fig 1 Clearance values and coefficients of excretion of lactate (O) and pyruvate (●) in living-donor transplants and cadaveric transplants Investigations were performed 0–1 day (*1*) 2–6 days (*2*) 7–20 days (*3*) 1–3 months (*4*) 3–12 months (*5*) and 12–14 months (*6*) after transplanta I=Standard error of $\bar{x}$ mean

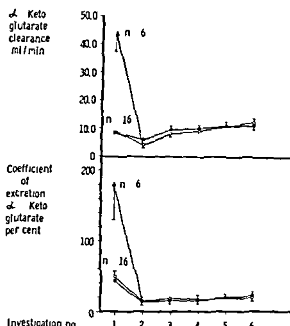


Fig 2 Clearance values and coefficients of excretion of α ketoglutarate in living-donor transplants (●) and cadaveric transplants (O) At the first investigation the cadaveric group was divided into one subgroup of 6 pa tients (Δ) and another subgroup of 16 patients (O) From investigation no 2 the cadaveric transplants are presented as one group (O) Investigation numbers and bars as in Fig 1

previously (2 4 5) Lactate pyruvate and α ketoglutarate were measured in blood and urine using enzymatic methods (6) Before the investigations all pa tients fasted for 8 hours whereupon renal clearances of lactate pyruvate and α ketoglutarate were determined concurrently with GFR and ERPF The fractional excre tions of the above mentioned substances are given as the ratio of the clearance values and [125]iothalamate clearance expressed in per cent For statistical calculations a Wilcoxon rank sum test was used

RESULTS

Group I (Figs 1 and 2) The serum concentrations of lactate pyruvate and α ketoglutarate were within the normal range at all investigations (3 6 8) Im mediately after transplantation the lactate clearance was significantly increased *(0 01<p<0 05)* com pared with values in unilaterally nephrectomized living donors and in patients with uremia as well (6) The coefficient of excretion was higher than in the donors but lower than in patients with uremia During the first 2–6 days lactate clearance de creased to a stable level within the same range as in unilaterally nephrectomized donors The pyruvate clearances did not differ significantly from those in donors neither did the coefficient of excretion (6) An insignificant decrease in pyruvate excretion was seen 2–6 days after transplantation

The α ketoglutarate excretion clearances and coefficients of excretion did not differ significantly from those in unilaterally nephrectomized donors with normal renal function (Fig 2) (6)

Group II In these patients the results for the excretional patterns of lactate and pyruvate did not differ from those in group I The coefficients of excretion were initially higher but renal function was poorer than in group I (Fig 1)

At the investigation immediately after transplan tation the α ketoglutarate excretion differed from *that in the other group This difference* emerged when group II was divided into two subgroups consisting of 6 and 16 patients The six patients had high clearance values of α ketoglutarate (Fig 2) The coefficient of excretion was increased to more than 100% indicating that secretion of α keto-

glutarate took place in these patients Their excretion of α ketoglutarate was reduced to the same values as in group I within 2–6 days after transplantation The other subgroup consisting of 16 patients did not differ from the patients in group I

One week after transplantation there were no differences in the excretional patterns of lactate pyruvate and α ketoglutarate between groups I and II and the values were within the same range as those obtained in donors with normal renal function after unilateral nephrectomy Investigations performed in 5 patients showed no significant changes in the excretional patterns of the above mentioned substances during rejection crises

DISCUSSION

Serum concentrations of lactate pyruvate and α ketoglutarate in transplanted patients did not differ from those in patients with normal renal function (6) and no variations were seen in the period from 2 hours to 14 months after transplantation

Immediately after transplantation a slight increase was observed in lactate excretion It is concluded that changes in lactate and pyruvate metabolism in the kidneys during ischemia are only modest or not reflected in the urine It must be born in mind however that a major part of the two substances is metabolized outside the kidneys (3 7) and that only small amounts of both lactate and pyruvate are found in urine from patients with normal renal function

In patients who had received kidneys with short ischemia times and good initial renal function the excretional patterns of α ketoglutarate resembled those of the normal controls (6) However in patients who had received cadaveric grafts with long ischemia times the excretion of α ketoglutarate did vary considerably In six patients the α ketoglutarate clearance exceeded the glomerular filtration rate indicating a net tubular secretion of this substance In the remaining 16 patients the average clearance of α ketoglutarate did not differ from the picture in donors with normal renal function That a net secretion of α ketoglutarate may take place is indicated by previous investigations (1 7) which have emphasized that α ketoglutarate is transported into the tubular cells by a mechanism similar to that transporting PAH and ^{131}I hippuran and secreted to the tubular lumen by a mechanism which has nothing to do with the PAH s transport The net secretion can be explained by an accumulation of α ketoglutarate in the kidney during the period of ischemia because of lack of oxygen If the secretory system is damaged there might be leakage of α ketoglutarate to the tubular lumen (1) This hypothesis could be supported by the finding that the high clearance of α ketoglutarate was short lasting and inconstant Any differences between the two groups of patients could not be detected by other means as renal function on an average was the same in the two groups (5)

Furthermore the clearance and coefficient of excretion for α ketoglutarate reached minimum values on days 2–6 after transplantation This could be explained by a high utilization of this compound in the restitution phase of the kidney

During acute rejection episodes no changes were observed in the excretional patterns of lactate pyruvate and α ketoglutarate

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Human Leucocyte Response to Migration Inhibitory Activity from Lymphocytes

Modification by Aprotinin Tranexamic Acid and Phenylmethyl Sulfonylfluoride

E Coeugniet B Søeberg and G Bendixen

From the Laboratory of Clinical Immunology and Medical Department TA Rigshospitalet University Hospital Copenhagen Denmark

ABSTRACT Human lymphokines can elicit several effects associated with inflammation e g leucocyte migration inhibition and fibrinolysis These effects can be assessed in vitro by the leucocyte migration agarose technique (LMAT) and the leucocyte migration fibrinolysis technique (LMFT) The present study shows that preincubation of normal leucocytes with aprotinin, tranexamic acid and phenyl methyl sulfonylfluoride (PMSF) reduces or abolishes their migration inhibition response to leucocyte migration inhibition factor The compounds exert this effect at non toxic concentrations, which do not otherwise interfere with migration or fibrinolysis, and are non toxic as estimated by PHA stimulation of lymphocytes The LMFT is more sensitive to the modifying effect than the LMAT The effect of aprotinin and tranexamic acid is reversible, the effect of PMSF is irreversible

The mechanism of leucocyte migration and lymphocyte dependent migration inhibition is insufficiently known and should be further studied to obtain information on the in vivo role of lymphokines in immunological inflammation The ability of leucocytes to migrate seems to be connected with the release of fibrinolytic activity and this interrelationship can be further examined in a system with standardized leucocyte migration in fibrin containing medium (6)

The present study reports the in vitro effect of antifibrinolytic/antiproteolytic compounds on leucocyte migration and the influence of such compounds on lymphokine induced leucocyte migration inhibition

MATERIAL AND METHODS

Tissue culture medium

TC medium 199 (Difco Michigan USA) with 10% horse serum (State Serum Institute Copenhagen) and penicillin and streptomycin at final concentrations of 67 IU/ml and 67 μg/ml respectively (TC 199)

Antifibrinolytic/antiproteolytic compounds

Aprotinin (APRO) (Novo Industrie GmbH Pharmaceutika Mainz Germany) Ampoules holding 10 ml containing 10 000 kallikrein inhibitor units (KIU)/ml (aqueous solution) Dilutions containing 1 000 100 and 10 KIU/ml in TC 199 were kept at −20°C until use

Tranexamic acid (TRAX) (Cyclokapron® Kabi Stockholm Sweden) Ampoules holding 5 ml containing 0 1 g/ml (aqueous solution) Dilutions containing 100 10 and 1 mg/ml in TC 199 were kept at −20°C until use

Phenyl methyl sulfonylfluoride (PMSF) (Sigma St Louis Mo USA and Merck Darmstadt Germany) A stock solution of PMSF was prepared by dissolving 174 mg powder (0 001 mol) in 1 ml *n* propanole followed by dilution 1 9 with distilled water Dilutions containing 1 74 mg/ml (10 mM) 0 174 mg/ml (1 mM) and 0 0174 mg/ml (0 1 mM) in TC 199 were kept at −20°C until use

Preparation of fibrin plates

Fibrin plates containing nutritive medium were prepared as follows 1 g human lyophilized fibrinogen (Kabi Stockholm Sweden) was dissolved in 100 ml distilled water 2 ml of the solution containing 20 mg fibrinogen was added to 0 9 ml tissue culture medium ten times concentrated (TC medium 199 10× (Difco Michigan USA)) 1 ml horse serum and 50 μl 10% sodium bicarbonate This mixture was diluted with 6 1 ml distilled water and penicillin and streptomycin were added to make final concentrations of 67 IU/ml and 67 μg/ml respectively Aliquots of 7 5 ml of this solution were transferred to plastic petri dishes 85 mm in diameter (Nunc Roskilde Denmark) and coagulated with 0 3 ml thrombin (50 NIH/ml) (Behringwerke

Table I *Direct effect of aprotinin (APRO) tranexamic acid (TRAX) and phenyl methyl sulfonyl fluoride (PMSF) on leucocyte migration*

MI direct~compound = mean of migration index in presence of compound LMAT = leucocyte migration agarose technique LMFT = leucocyte migration fibrin technique

	Concentration	MI direct~compound	
		LMAT*	LMFT*
APRO	1 000 kIU/ml	0 87±0 14	0 87±0 1
	100	1 03±0 1	1 06±0 14
	10	0 99±0 14	1 05±0 14
	1	0 94±0 10	0 96±0 05
TRAX	10 000 μg/ml	0 95±0 10	0 89±0 10
	1 000	1 0 ±0 1	0 97±0 10
	100	0 97±0 07	0 95±0 06
	10	0 98±0 14	1 0 ±0 07
	1	1 0 ±0 09	0 93±0 04
PMSF	100 mM	0 94±0 1	0 87±0 1
	10	1 02±0 06	1 04±0 13
	1	0 90±0 1	1 03±0 2
	0 1	0 96±0 05	1 01±0 15

* Non-significant differences (Wilcoxon s test)

Magdeburg Germany) The plates were left for coagulation at room temperature for 30 min and then used

Preparation of agarose plates

Agarose plates were prepared as described in detail by Clausen (5) Briefly a 1% solution of agarose in TC 199 is ... to plastic petri dishes When the medium has solidified cylindrical wells to contain the cell suspensions are punched in the gel

Preparation of culture supernatants containing leucocyte migration inhibitory activity (LMIA)

LMIA was produced by stimulation with concanavalin A (Con-A) of separated 95% pure mononuclear cells from human peripheral blood as described elsewhere (1) Biologically demonstrable Con-A activity and components of TC 199 were removed by Sephadex column passage as previously described in detail (1) leaving a final product of cell free lyophilized active supernatant (AS) of Con-A stimulated human mononuclear cells and a similar preparation of control supernatant (CS) of comparable non-stimulated human mononuclear cells All the following experiments were performed in parallel with AS and CS

Assessment of LMIA

20×10^6 thrice washed normal human leucocytes (indicator cells) were suspended in 1) 100 μl TC 199 2) 100 μl AS and 3) 100 μl CS In parallel indicator cells were suspended in TC 199 AS and CS containing dilutions of APRO TRAX and PMSF at final concentrations as indicated in Table I The cells were incubated at 37°C for 60 min and applied in parallel on agarose and fibrin plates The transfer of cell suspension to the wells on the agarose plate has been described previously in detail (5) The application on the fibrin plates was made as follows Aliquots of 7 μl of the different cell suspensions were applied with micropipettes on each plate (H Pedersen Copenhagen Denmark) carefully so as to place spherical drops on fibrin areas of equal size Agarose plates and fibrin plates were incubated at 37°C in a 2% carbon dioxide and water saturated atmosphere for 22 hours and the

Table II *Effect of aprotinin (APRO) tranexamic acid (TRAX) and phenyl methyl sulfonylfluoride (PMSF) on leucocyte response to lymphokine-containing supernatants (AS)*

MI~control = leucocyte migration inhibitory activity of AS without addition of compound MI~compound = leucocyte migration inhibitory activity of AS influenced by APRO TRAX or PMSF LMAT = leucocyte migration agarose technique LMFT = leucocyte migration fibrin technique

	Concentration	MI~control ± S D		MI~compound ± S D		% inhibition of leucocyte response to AS	
		LMAT	LMFT	LMAT	LMFT	LMAT	LMFT
APRO	1 000 kIU/ml			0 97±0 05	0 98±0 07	87*	92*
	100	0 78±0 03	0 75±0 08	0 81±0 08	0 94±0 05	n s.	76
	10			0 69±0 07	0 67±0 06	n s	n s
	1			0 69±0 07	0 72±0 07	n s	n s
TRAX	10 000 μg/ml			0 89±0 04	0 94±0 05	50*	76
	1 000			0 80±0 10	0 90±0 03	n.s	60*
	100	0 78±0 03	0 75±0 08	0 76±0 07	0 72±0 06	n s	n s.
	10			0 74±0 11	0 76±0 03	n s	n s
	1			0 80±0 08	0 72±0 10	n s	n s
PMSF	100 mM			0 99±0 09	1 02±0 04	96	100
	10	0 78±0 03	0 75±0 08	0 94±0 05	1 05±0 05	73*	100*
	1			0 81±0 08	0 90±0 03	n s	60*
	0 1			0 69±0 07	0 68±0 08	n s.	n s

* $p<0\ 05$ n.s. = not significant (Wilcoxon s test)

Table III *Modification by aprotinin (APRO) tranexamic acid (TRAX) and phenyl methyl sulfonylfluoride (PMSF) of ^{14}C thymidine incorporation in non stimulated and PHA stimulated lymphocytes (mean ±S E M)*

	Concentration	Non stimulated lymphocytes *(cpm)*	PHA stimulated lymphocytes *(cpm)*
Control		57+22	13 555±130
APRO	2 000 KIU/ml	66±10	9 683±1 434
	200	94±29	13 193±911
	20	76±13	12 727±52
	2	39+11	13 358±393
TRAX	20 000 μg/ml	70±23	10 884±183
	2 000	64+24	13 991±1 359
	200	110±18	13 941±1 776
	20	80+7	13 390±394
	2	82+9	13 785±699
PMSF	10 mM	7+5	31±15
	1	52±19	12 108±673
	0 1	75±8	13 312±1 586
	0 01	35+9	11 614±1 242
	0 001	50±9	13 469±1 175

migration areas of cells were measured in a projection microscope The LMIA was expressed as a migration index (MI)

$$MI = \frac{\text{mean of migration areas of leucocytes suspended in AS}}{\text{mean of migration areas of leucocytes suspended in CS}}$$

The influence of APRO TRAX and PMSF on LMIA was calculated by comparing the MI in the presence of each compound (MI~compound) with the MI in the absence of the compound (MI~control) according to the formula

$$\%\ \text{inhibition of LMIA} = \frac{(\text{MI}\sim\text{compound})-(\text{MI}\sim\text{control})}{1-(\text{MI}\sim\text{control})}\times 100$$

A possible direct effect of APRO TRAX and PMSF on leucocyte migration was expressed in the following way

$$\text{MI direct}\sim\text{compound} = \frac{\text{mean migration areas in TC 199 with compound}}{\text{mean migration areas in pure TC 199 control}}$$

The assays were made in parallel and simultaneously in the agarose and fibrinolysis systems

Assays for toxic effect of APRO TRAX and PMSF on cells

Influence on blast transformation and on trypan blue exclusion Cultures of 95% pure mononuclear cells were obtained from peripheral blood of healthy adults by centrifugation in Ficoll isopaque (Lymphoprep® Nyegaard Oslo Norway) and resuspended in RPMI 1640 (Gibco Grand Island NY USA) supplemented with 15% pooled human serum at 5×10^5 cells in 500 μl

Blast transformation test

Cultures were done in triplicate with the following groups in parallel 1) Unstimulated cultures in medium alone 2) Same but APRO TRAX and PMSF added in ten fold dilutions 3) Mitogen stimulated cultures with phytohemagglutinin *(PHA) (Difco Michigan USA) 40* μg/ml added per culture 4) Same but APRO TRAX and PMSF added in tenfold dilutions

After 48 hours of incubation 50 μl of ^{14}C thymidine 0 1 μCi was added to each culture the culture was continued for 24 hours and the cells were harvested for determination of ^{14}C thymidine incorporation expressed as cpm

Trypan blue exclusion test

The final concentration of trypan blue was 0 2% Cells were incubated with or without APRO TRAX and PMSF and assayed after 4 and 22 hours of incubation

RESULTS

The findings are presented in Tables I and II Both by leucocyte migration agarose technique (LMAT) and leucocyte migration fibrin technique (LMFT) APRO TRAX and PMSF at the highest concentrations significantly reduced the leucocyte response to AS but did not by themselves significantly modify the migration areas A tenfold dilution of APRO *TRAX and PMSF resulted in abolition of the effect* as assessed by the LMAT but in the LMFT APRO and PMSF still significantly reduced the leucocyte migration response to AS The lowest concentrations of APRO TRAX and PMSF did not modify the leucocyte response to AS neither in the LMAT nor the LMFT

Cell viability

The influence of APRO TRAX and PMSF on blast transformation is shown in Table III PMSF at 10 mM markedly depressed the stimulation by PHA but at all concentrations used in the LMAT and LMFT the compounds did not significantly influence the PHA induced lymphocyte transformation and did not stimulate the unstimulated cultures No change in leucocyte viability as assayed by the trypan blue exclusion test was found with the highest concentrations used of APRO TRAX and PMSF

Parallel studies in the same experimental models showed that the final concentrations of *n* propanole (solvent for PMSF) obtained in the individual experiments were non toxic to the cells

DISCUSSION

APRO is a polypeptide with enzyme inhibiting activity The compound is a kallikrein trypsin inactivator which is used in the treatment of acute pancreatitis and it also inhibits the activity of plasmin and the activation of plasminogen in fibrinolytic states (18) Its antiproteolytic activity in vivo and in vitro has been shown in multiple experimental models (4 7 9 10 11 12 13 15 16 18 19) It is of interest in this context that APRO inhibits the release of proteolytic activity from granulocytes and modifies their dynamics (10) Because of the broad spectrum of activities of APRO and especially because of its antifibrinolytic activity we decided to examine the influence of APRO on in vitro leucocyte migration both in a fibrin containing and in a fibrin free medium A priori one might expect APRO in some way to modify the migratory capacity of leucocytes since the substance has an enzyme inhibiting activity and since observations suggestive of this idea were made in vivo in animal models (10) A similar modification might cause alteration of the leucocyte response to lymphokines With the experimental system used however an expected change in migratory ability of leucocytes measured with LMAT as well as LMFT is induced only by high clinically non relevant con[illegible] ations of APRO

The ability of antifibrinolytic/antiproteolytic sub[illegible]ces to decrease the response of leucocytes to AS was evident in both LMAT and LMFT at high concentrations Lower concentrations influenced the MI only in the LMFT This observation could be an argument for an interdependence between migration migration inhibition lymphokine effect and proteolytic activity since migration cultures in a medium that includes a step for assessment of the proteolytic effect (fibrin containing medium) is more sensitive than a fibrin free medium

TRAX is a synthetic antifibrinolytic compound with an effect 10–100 times that of epsilon aminocaproic acid The drug has a low toxicity and its antienzymatic activity is manifested by competitive inhibition of urokinase streptokinase and plasmin activities (17)

The sulfonylester PMSF is highly toxic in vivo since it causes an irreversible inhibition of esterase e g α-chymotrypsin and trypsin (8) PMSF inhibits LMIA irreversibly (2 3)

In the present experiment APRO TRAX and PMSF were all shown to inhibit the effect of [illegible] sufficiently high doses were used There is n[illegible] dence however that this ability is due to a [illegible] toxic effect since at this concentration of the [illegible] pounds no influence was seen on viability as ju[illegible] by trypan blue exclusion test the transform[illegible] response to PHA or migrating ability in c[illegible] medium with or without compounds

The individual mode of action of APRO T[illegible] and PMSF probably even differs as the inhib[illegible] effect of PMSF could not be dialysed away in[illegible] trast to that of APRO and TRAX (unpubli[illegible] observation) APRO and TRAX influence le[illegible] cytes to respond by a direct and a transitory e[illegible] on the cells since, after being washed the le[illegible] cytes recover their ability to respond to AS

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Lactoferrin in Human Neutrophilic Polymorphonuclear Leukocytes in Relation to Iron Metabolism

B J C M de Vet and C H ten Hoopen

From the Department of Experimental Internal Medicine Wilhelmina Gasthuis University of Amsterdam The Netherlands

ABSTRACT Lactoferrin (LF), the iron binding protein of external secretions and neutrophilic polymorphonuclear leukocytes (PMN), was studied in 27 patients during granulocytosis caused by acute inflammation and in disorders without granulocytosis (iron deficiency anemia, iron overload and liver dis eases) During granulocytosis the LF concentration of PMN was significantly lower than in controls ($p<0\ 001$) This difference proved to be related to the number of PMN A relation between the LF concen tration of PMN and iron metabolism could be demon strated loss of iron by blood donation is accompanied by a significant decrease in the LF concentration in PMN, whereas iron therapy in patients with iron deficiency anemia is accompanied by a significant increase in the LF concentration in PMN

Lactoferrin (LF) has been demonstrated in many biological fluids such as milk tears bile pancreat ic duodenal and synovial fluid in man as well as in other species (2 8 21 22 24) Like transferrin (TF) LF is able to bind reversibly two atoms of iron Under physiological circumstances its satura tion with iron is only 20%

For LF as well as for TF two functions have been established 1) Iron free LF (apo-LF) shows in vitro bacteriostatic properties which are lost if LF is saturated with iron (6 14 21 32) 2) LF plays a role in iron absorption (4 5 15 33 34 35) In humans with normal iron metabolism LF has an inhibitory effect on iron absorption in the small intestine LF has no such effect in patients with idiopathic hemosiderosis and causes only a slight inhibition of iron absorption in patients with hepatic cirrhosis Moreover LF concentrations of duo denal fluids in patients with hemosiderosis and cirrhosis are very low (33 34)

Besides in external secretions LF has been identified in neutrophilic polymorphonuclear leu kocytes (PMN) of humans rats and rabbits (1 11 12 16 17 23 26 28 32) Erythrocytes platelets basophilic and eosinophilic leukocytes do not contain LF In vitro human blood monocytes can fix and ingest LF as shown by van Snick et al (31) Plasma concentrations of LF are very low (normal value <1 μg/ml) This plasma LF is as far as is known released from PMN (12 13 17 18 27 28 30) Although there has been some discussion about the presence of LF either in the nucleus (11) or in the cytoplasmic granules of PMN it has now been established that LF is present in the specific secondary granules of PMN starting with the pro-myelocytic stage (1 16 23) LF is considered to be the most specific marker for the secondary gran ules comparable to myeloperoxidase for the primary azurophilic granules (17) During phago-cytosis the granule proteins are released into the phagosomes being involved with the intracellu lar killing of micro-organisms As degranulation of secondary granules occurs already before immune complexes or micro-organisms are completely en closed by the PMN leakage of LF into the sur rounding medium is possible (17)

The significant positive relation between the total blood granulocyte pool and the plasma LF concen tration as postulated by Hansen et al (13) sup-ports the assumption that LF in the plasma origi nates from PMN However highly increased plas ma levels have been found only in patients with chronic myelocytic leukemia (13 18 28) During the acute phase of inflammation only a slight in crease in the plasma LF concentration to 3 times the normal values is observed (12) This might be

Table I *Hematological data (mean±S D)*

Patient group	n	Hb (μmol/l)	Hct	Fe (μmol/l)	TIBC (μmol/l)	Saturation (%)	Leukocytes (×10³/mm³)
Controls	36	9 4±0 7	0 45±0 03	20 1±6 6	61 0± 8 2	33 7±10 6	2 88±0 94
Iron deficiency anemia	4	6 6±0 4	0 34±0 03	8 1±3 9	59 3±21 1	17 0±10 3	3 71±1 .8
Hemochromatosis*	4	8 9±0 4	0 42±0 02	27 4±4 9	48 3±15 9	64 8±15 9	4 54±1 99
Hepatic cirrhosis	7	7 9±1 3	0 38±0 06	15 1±7 0	62 8± 9 9	24 9±11 8	3 36±1 07
Acute hepatitis*	3	9 8±0 7	0 47±0 04	49 4±5 9	63 5± 3 1	77 7± 5 8	2 58±0 88
Acute inflammation	9	8 3±1 5	0 42±0 06	5 7±3 1	44 4±11 8	13 9± 5 8	17 26±9 20

* Proved by liver biopsy

due to the rapid clearance of LF from the blood by the macrophage system during infection as shown by van Snick et al (31)

As apo-LF inhibits in vitro the growth of bacteria (6 22) and fungi (14) it is highly possible that apo-LF in PMN plays a role in intracellular killing of micro-organisms Absence lowered concentrations or a defective LF might be the cause of decreased bactericidal or fungicidal activity Low LF concentrations of PMN are described in chronic granulocytic leukemia (26) but until now only one case has been published in which LF deficiency of PMN might be related to a serious bacterial infection (32)

There has been much speculation about the relation between iron deficiency anemia and the ...ity to bacterial infections (9) We there... investigated the LF concentration of PMN in ...llowing conditions 1) During granulocytosis in acute inflammations 2) in iron deficiency anemia and iron overload 3) in hepatic diseases as cirrhosis and acute hepatitis in which severe abnormalities of iron metabolism can be found

METHODS

Isolation of human LF and preparation of monospecific LF antibody

LF was isolated from human milk by centrifugation separating fat and casein followed by ammonium sulphate precipitation and column chromatography as described previously (33 34) Antiserum against LF was prepared in rabbits by 4 i m injections of 0 5 mg LF administered at intervals of 14 days After absorption with pooled human serum contaminating antibodies were no longer detectable (33 34)

Preparation of a leukocyte concentrate

Human leukocytes were separated from red cells by accelerated sedimentation of the latter by means of dextran (M 500000) as described by Masson et al (21 23) with a few modifications After centrifugation at 500 g the leukocyte pellet is resuspended in 0 1 M phosphate buffer + 1 0 M sodium chloride pH 8 0 The cells are disrupted by freezing and thawing 6 times (−20°C +37°C) and LF is determined in the supernatant after centrifugation at 750 g The PMN are counted in the leukocyte concentrate in order to calculate the LF concentration per 10⁶ PMN

Immunohistochemical techniques

Indirect immunofluorescent staining Citrate anticoagulated buffy coat smears are dried and fixed by means of absolute methanol for 3 min followed by incubation for 30 min with LF antiserum (absorbed with erythrocytes of the same person and rabbit liver powder dilution 1 240) and fluorescein isothiocyanate labeled goat antirabbit Ig (dilution 1 150) All steps were carried out at room temperature Following each stage smears were washed for 30 min in phosphate buffered saline pH 7 4

Immunoperoxidase staining direct method (25) EDTA anticoagulated buffy coat smears are dried and fixed for 30 sec by means of buffered formol acetone (20) After fixation slides are processed as follows Blocking of the endogenous peroxidase in absolute methanol containing 0 3% hydrogen peroxidase for 1 hour Incubation with peroxidase-conjugated LF antiserum for 1 hour Development of the brown colour with DAB=3 3 diamino-*benzidinetetrahydrochloride* (0 6 mg/ml phosphate buffered saline pH 7 4) and hydrogen peroxide (0 01%) for 8 min (10) Counterstaining with hematoxylin Following each stage smears were washed for 30 min in phosphate buffered saline pH 7 4

Iron determination

The plasma iron concentration and total iron binding capacity (TIBC) were estimated with the iron test combination of Boehringer Mannheim West Germany

Statistical methods

Differences in LF concentrations numbers of leukocytes and iron concentrations were tested for statistical significance by Student s *t* test (7)

PATIENTS

Six groups of patients were studied The laboratory data are summarized in Table I

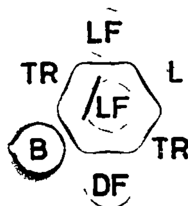


Fig 1 Immunodiffusion in agar *LF*=purified human lactoferrin *L*=leukocyte rich extract *TR*=tears *DF*=duodenal fluid *B*=bile */LF*=rabbit antiserum against human LF The precipitation lines show immunological identity



Fig 3 Direct immunoperoxidase staining of PMN *(a)* Diffuse cytoplasmic staining of PMN which is absent in mononuclear cells *(b)* After absorption of the LF antiserum with purified LF specific cytoplasmic staining of PMN is absent but non specific staining of eosinophilic leukocytes remains

RESULTS

Qualitative determinations

LF was identified in extracts of leukocyte rich samples by means of gel immunodiffusion (29) Complete fusion was observed of the precipitation lines of the leukocyte extract with LF of tears bile duodenal fluid and purified LF isolated from human milk (Fig 1)

Immunohistochemical studies

Indirect immunofluorescence of PMN showed a bright cytoplasmic staining (Fig 2) After absorption of the antiserum with purified LF the fluorescence of PMN was abolished We did not find a nuclear localization of LF as described by Green et al (11) Erythrocytes monocytes and lymphocytes showed no fluorescence whereas eosinophilic leukocytes retained their autofluorescence even after absorption of the LF antiserum with LF



Fig 2 Cytoplasmic fluorescence of PMN No nuclear localization of LF Indirect immunofluorescent technique

With a direct immunoperoxidase technique all PMN showed diffuse cytoplasmic staining being absent in monocytes lymphocytes and thrombocytes Erythrocytes stained faintly on account of their high endogenous peroxidase activity (Fig 3) Eosinophilic leukocytes showed some non specific staining remaining after absorption of the LF antiserum with purified LF It is however very easy to distinguish eosinophilic leukocytes and PMN by the nuclei and the different cytoplasmic colour

Quantitative determination

LF was measured in the leukocyte concentrate by the single diffusion test (19) The LF concentration (μg/10^6 PMN) was calculated from

$$\frac{\text{amount of LF/ml leukocyte concentrate}}{\text{number of PMN/ml leukocyte concentrate}}$$

The control group (30 men and 6 women) had a *mean LF concentration±S D of* 7 7±1 9 $\mu g/10^6$ PMN There were no statistically significant differences between the sexes

Two of the control leukocyte concentrate samples were divided into 5 portions of 1 ml in each of which the LF concentrations and the numbers of PMN were measured separately The mean LF concentrations of the two samples were 5 4 and 7 4

Table II *LF concentration of PMN in patients and controls*

	LF concentration (μg/10⁶ PMN)			
Patient group	Mean	S E M	S D	Range
Controls	7 7	0 3	1 9	5 5–13 2
Iron deficiency anemia	6 3	0 2	0 4	5 8– 6 9
Hemochromatosis	6 8	0 8	1 4	5 8– 9 0
Hepatic cirrhosis	7 3	0 5	1 2	5 8– 9 0
Acute hepatitis	7 7	1 7	2 4	5 4–11 0
Acute inflammation	*4 8*	*0 5*	*1 3*	*3 0– 7 6*

μg/10⁶ PMN respectively In both cases the S D of *the determination appeared to be very small (0 2)* In 8 control leukocyte extracts LF was measured before and after storage at −20°C for one month LF concentrations after storage did not differ significantly from the fresh samples (t=1 5 p> 0 1)

Repeated estimation of LF concentrations in PMN of one and the same patient at weekly intervals showed that LF concentrations fluctuate within narrow limits (range 6 2–6 9 μg/10⁶ PMN mean 6 5±0 2)

LF concentrations of PMN were studied in 5 *groups of patients The hematological data are* summarized in Table I The results were compared with the normal values These data are presented in e II and Fig 4

The mean LF concentration of PMN is significantly lower in patients with acute inflammation than in controls (t=5 3 p<0 001) although some overlap exists This difference proved to be related to the number of PMN per ml blood as we found a striking negative correlation between the number of *PMN and the LF concentration of PMN* (r=−0 53 p<0 001) As the total number of PMN per ml blood is significantly increased in these patients total LF *in all PMN of 1 ml blood is the same as in normal* controls or even higher

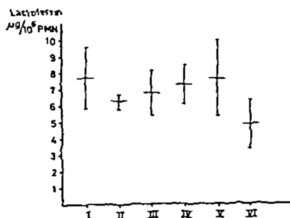


Fig 4 LF concentration of PMN (mean±S D) *I*=controls (n=36) *II*=patients with iron deficiency anemia (n=4) *III*=patients with hemochromatosis (n=4) *IV*=patients with hepatic cirrhosis (n=7) *V*=patients with acute hepatitis (n=3) *VI*=patients with granulocytosis as a result of acute inflammation (n=9)

Talbe III *Effect of blood donation on the LF concentration of PMN*

	LF concentration (μg/10⁶ PMN)	
Donor no	Before blood donation	10 days after blood donation
1	8 4	5 3
2	7 8	5 0
3	8 3	5 5
4	10 1	10 4
5	7 8	7 6
6	7 0	2 8
7	7 6	6 4
8	10 2	6 7
9	8 8	7 0
10	6 4	6 6
11	9 6	9 0
12	13 3	9 0
13	9 3	8 2
Mean	8 8	6 9
S E M	0 5	0 6
S D	1 7	1 9

As inflammations are often accompanied by disturbances of iron metabolism we studied in more *detail the relation between the LF concentration of* PMN and iron metabolism in controls in patients with iron deficiency and iron overload and in patients with liver diseases (acute hepatitis and hepatic cirrhosis) in whom also alterations of iron metabolism are frequently found Although none of these groups displayed a direct relation between the LF concentration of PMN and parameters of iron metabolism (plasma iron TIBC and Fe saturation) it is of interest that the mean LF concentration was significantly lower in patients with iron deficiency anemia (without granulocytosis) than in controls (t=3 8 p<0 001) The LF concentrations of PMN in patients with iron overload and liver diseases showed no significant differences from the controls although plasma iron was very high especially in acute hepatitis

In order to investigate the effect of iron loss or

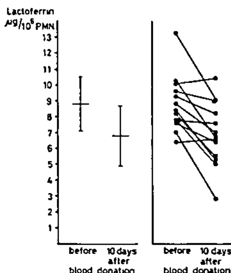


Fig 5 LF concentration of PMN of 13 donors before and 10 days after blood donation Mean values ±S D to the left individual values to the right There is a highly significant difference between the mean LF concentrations on the two days ($p<0.005$)

iron therapy on the LF concentration of PMN we studied the following two groups of patients

In 13 blood donors LF concentrations of PMN were measured just before and 10 days after blood donation The results are presented in Table III and Fig 5 The concentrations decreased in 10 of the 13 donors The difference between the mean LF con

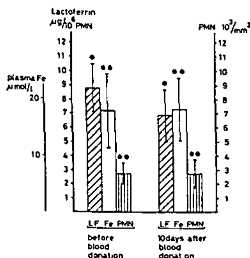


Fig 6 LF concentration plasma iron concentration and number of PMN of 13 donors before and 10 days after blood donation (mean±S D) * Statistically significant difference ($p<0.005$) ** not significant

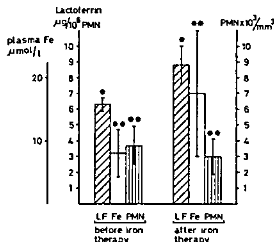


Fig 7 LF concentration plasma iron concentration and number of PMN of 4 patients with iron deficiency anemia before and after iron therapy (mean±S D) * Statistically significant difference ($p=0.01$) ** not significant

centrations on these two days is highly significant ($t=4.29$ $p<0.005$) Plasma iron concentrations and numbers of PMN showed no differences (Fig 6) In 4 donors LF concentrations of PMN were also measured on the 4th and 7th day after blood donation The lowest level was mostly reached on the 4th day after donation increasing afterwards

In 4 patients with iron deficiency anemia the effect of iron therapy on LF concentrations plasma iron and numbers of PMN was studied The results are presented in Fig 7 LF concentrations of PMN increased significantly in all 4 ($t=3.4$ $p=0.01$) As expected the plasma iron concentrations also increased in all patients The mean number of PMN showed no significant alterations

DISCUSSION

In accordance with Masson et al (21 23) and Mason et al (20) LF could be detected in the cytoplasm of mature PMN and bands using immunofluorescent and immunoperoxidase techniques Masson et al (23) showed that LF appears during the promyelocytic stage and that cytoplasmic concentration increases until the stage of mature PMN The specific localization of LF in the nuclei of PMN as reported by Green et al (11) can be ascribed to an artifact due to fixation techniques in which ethanol has been used instead of methanol

Only a few investigators measured LF in PMN

quantitatively The results were as follows Masson et al (21 23) 3 4±0 3 μg/10⁶ PMN Rumke et al (30) 5 0 Leffell and Spitznagel (16) 6 4±1 8 Olofsson et al (26) 4 4±1 2 Hansen et al (13) 1 82 μg/10⁶ PMN We found a higher value (7 7± 1 9 μg/10⁶ PMN) in our control group The reason for this is not clear but can be due to slight differences in technique

As shown by Hansen et al (13) low plasma LF concentrations correlate significantly with the total number of PMN in the peripheral blood and with the turnover rate of PMN which indicates that PMN are the main source of plasma LF Recently Hansen et al (12) studied LF concentrations of PMN and plasma during acute inflammation and found that the concentrations during the acute inflammatory phase were lower than in the convalescent stage Plasma LF concentrations during the acute phase however amounted to 3 times the normal values In accordance with these observations we found a significantly lower LF concentration of PMN during the acute phase of inflammation The low LF concentration of PMN during the granulocytosis of acute inflammation might be explained by leakage of LF to the plasma On the other hand LF in the PMN often remains very low when the infection subsides and plasma LF is normalized again (12) It is also quite possible LF synthesis in the bone marrow is decreased ' are available on this problem

second part of our investigation concerned relation between LF of PMN and iron metabolism Although in individual patients no correlation was found between the LF concentration of PMN and parameters of iron metabolism loss of iron (donation of 450 ml blood) proved to be associated with a significant decrease in LF concentrations of PMN whereas iron therapy in patients with iron deficiency anemia resulted in significantly increased concentrations

From our results it may be concluded that LF in PMN depends on iron metabolism Iron deficiency seems to be accompanied by low LF concentrations and iron therapy augments LF concentrations of PMN The lowest LF concentrations of PMN were observed in acute inflammation possibly as a combination of iron deficiency and granulocytosis

These findings need not conflict with the theory of Bennett et al (3) van Snick et al (31) and Hansen et al (12) suggesting that during inflammation iron sequestration in the RES occurs due to macrophages taking up a LF–Fe complex in which the iron is derived from transferrin LF has a stronger binding capacity to iron especially with lower pH as occurs in the inflammatory area

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Table II *Results of the ¹³¹I labelled study*

Group 1=treated with clofibrate group 2=controls
EVAS=extravascular albumin space EVBrS=extravascular bromide space

Case no	Intra vascular albumin mass (g)	Extra vascular albumin mass (g)	Distri bution ratio (%)	Rate of albumin synthesis (g/d)	Fractional catabolic rate (%)	Half time of the first exponential slope ($t_{1/2}$) (d)
Group 1						
10	173	290	37 4	24 6	14 2	0 85
12	115	167	40 7	15 5	13 5	1 19
17	99	187	34 6	11 0	11 2	1 16
18	136	177	43 4	13 4	9 8	0 98
20	89	120	42 5	12 3	13 8	1 03
24	116	161	41 9	14 1	12 2	0 81
25	79	107	42 5	9 7	12 3	1 20
Mean	115	173	40 4	14 4	12 4	1 03
S D	31 7	59 5	3 24	4 91	1 56	0 161
Group 2						
4	145	187	43 7	15 1	10 4	0 95
5	158	175	47 5	16 4	10 4	0 83
7	111	148	42 9	11 8	10 6	0 94
9	115	175	39 6	16 4	14 3	0 75
10	146	240	38 0	19 0	13 0	0 79
11	102	110	48 2	9 4	9 2	1 17
12	98	131	42 8	12 2	12 5	0 76
13	118	134	46 8	16 1	13 7	0 85
14	130	151	46 2	17 0	13 1	0 89
17	114	158	42 0	12 9	11 3	0 94
1	116	143	44 9	14 8	12 7	0 91
2	101	121	45 6	11 7	11 6	0 84
3	151	177	46 1	14 9	9 9	0 87
8	173	199	46 5	19 0	11 0	0 83
23	110	132	45 4	9 2	8 3	1 02
	126	159	44 4	14 4	11 5	0 89
	23 2	34 0	2 81	3 09	1 72	0 108
s *t* test *p* value	>0 3	>0 5	<0 01	–	>0 2	<0 05

$I/\Sigma c/b$ *Rate of albumin synthesis* (mass of albumin synthesized per day) $IVM/\int Pdt$ or $IVM/\Sigma c/b$ *Distribution ratio* (*IVM/TM*) $(\Sigma c/b)^2/\Sigma c/b^2$ *Albumin space* TM/albumin concentration in serum (mean) *Apparent Br space* $(ads - vu)/1\,000 \times p$ (*a* is the volume of injected dose (ml) *d* the dilution factor of standard *s* the count rate of diluted standard *u* the count rate of urine sample/ml *v* the volume of collected urine during 24 hours and *p* the count rate of plasma sample drawn 24 hours after the dose is given) *Extravascular albumin space* Albumin space–plasma volume *Extravascular bromide space* 24 hours bromide space–plasma volume The results were compared by Student s *t* test

RESULTS

The main results including 1 S D and the *p* values from Student s *t* test, are given in Table II and Fig 1 They show the following significant differences between the two groups

The distribution ratio is smaller in the clofibrate treated subjects (group 1) than in the controls (group 2) (group 1 40 4±3 24% group 2 44 4 ±2 81% $p<0\ 01$) the half time of the first exponential slope ($t_{1/2}$) is longer (group 1 1 03±0 16 days group 2 0 89±0 11 days $p<0\ 05$) and the extravascular albumin space calculated as a percentage of the bromide space is greater in the treated than in the control group (group 1 22 1±1 54% group 2 19 6±1 49% $p<0\ 01$) (Fig 1)

The drug did not cause any significant change in the rate of albumin synthesis (group 1 14 4±4 91

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Lactoferrin in Human Neutrophilic Polymorphonuclear Leukocytes in Relation to Iron Metabolism

B J C M de Vet and C H ten Hoopen

From the Department of Experimental Internal Medicine Wilhelmina Gasthuis University of Amsterdam The Netherlands

ABSTRACT Lactoferrin (LF), the iron binding protein of external secretions and neutrophilic polymorphonuclear leukocytes (PMN), was studied in 27 patients during granulocytosis caused by acute inflammation and in disorders without granulocytosis (iron deficiency anemia, iron overload and liver diseases) During granulocytosis the LF concentration of PMN was significantly lower than in controls ($p<0.001$) This difference proved to be related to the number of PMN A relation between the LF concentration of PMN and iron metabolism could be demonstrated loss of iron by blood donation is accompanied by a significant decrease in the LF concentration in PMN, whereas iron therapy in patients with iron deficiency anemia is accompanied by a significant increase in the LF concentration in PMN

Lactoferrin (LF) has been demonstrated in many biological fluids such as *milk tears bile pancreatic* duodenal and synovial fluid in man as well as in other species (2 8 21 22 24) Like transferrin (TF) LF is able to bind reversibly two atoms of iron Under physiological circumstances its saturation *with iron is only 20%*

For LF as well as for TF two functions have been established 1) Iron free LF (apo LF) shows in vitro bacteriostatic properties which are lost if LF is saturated with iron (6 14 21 32) 2) LF plays a role in iron absorption (4 5 15 33 34 35) In humans with normal iron metabolism LF has an inhibitory effect on iron absorption in the small intestine LF has no such effect in patients with idiopathic hemosiderosis and causes only a slight inhibition of iron absorption in patients with hepatic cirrhosis Moreover LF concentrations of duodenal fluids in patients with hemosiderosis and cirrhosis are very low (33 34)

Besides in external secretions LF has been identified in neutrophilic polymorphonuclear leukocytes (PMN) of humans rats and rabbits (1 11 12 16 17 23 26 28 32) Erythrocytes platelets basophilic and eosinophilic leukocytes do not contain LF In vitro human blood monocytes can fix and ingest LF as shown by van Snick et al (31) Plasma concentrations of LF are very low (normal value <1 μg/ml) This plasma LF is as far as is known released from PMN (12 13 17 18 27 28 30) Although there has been some discussion about the presence of LF either in the nucleus (11) or in the cytoplasmic granules of PMN it has now been established that LF is present in the specific secondary granules of PMN starting with the promyelocytic stage (1 16 23) LF is considered to be the most specific marker for the secondary granules comparable to myeloperoxidase for the primary azurophilic granules (17) During phagocytosis the granule proteins are released into the phagosomes being involved with the intracellular killing of micro-organisms As degranulation of secondary granules occurs already before immune complexes or micro-organisms are completely enclosed by the PMN leakage of LF into the surrounding medium is possible (17)

The significant positive relation between the total blood granulocyte pool and the plasma LF concentration as postulated by Hansen et al (13) supports the assumption that LF in the plasma originates from PMN However highly increased plasma levels have been found only in patients with chronic myelocytic leukemia (13 18 28) During the acute phase of inflammation only a slight increase in the plasma LF concentration to 3 times the normal values is observed (12) This might be

Table I *Hematological data (mean ± S D)*

Patient group	*n*	Hb (μmol/l)	Hct	Fe (μmol/l)	TIBC (μmol/l)	Saturation (%)	Leukocytes (×10³/mm³)
Controls	36	9 4±0 7	0 45±0 03	20 1±6 6	61 0± 8 2	33 7±10 6	2 88±0 94
Iron deficiency anemia	4	6 6±0 4	0 34±0 03	8 1±3 9	59 3±21 1	17 0±10 3	3 71±1 28
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* Proved by liver biopsy

due to the rapid clearance of LF from the blood by the macrophage system during infection as shown by van Snick et al (31)

As apo-LF inhibits in vitro the growth of bacteria (6 22) and fungi (14), it is highly possible that apo LF in PMN plays a role in intracellular killing of micro-organisms Absence lowered concentrations or a defective LF might be the cause of decreased bactericidal or fungicidal activity Low LF concentrations of PMN are described in chronic granulocytic leukemia (26) but until now only one case has been published in which LF deficiency of PMN might be related to a serious bacterial infection (32)

There has been much speculation about the relation between iron deficiency anemia and the ity to bacterial infections (9) We there investigated the LF concentration of PMN in f wing conditions 1) During granulocytosis in acute inflammations ?) in iron deficiency anemia and iron overload 3) in hepatic diseases as cirrhosis and acute hepatitis in which severe abnormalities of iron metabolism can be found

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Isolation of human LF and preparation of monospecific LF antibody

LF was isolated from human milk by centrifugation separating fat and casein followed by ammonium sulphate precipitation and column chromatography as described previously (33 34) Antiserum against LF was prepared in rabbits by 4 i m injections of 0 5 mg LF administered at intervals of 14 days After absorption with pooled human serum contaminating antibodies were no longer detectable (33 34)

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Human leukocytes were separated from red cells by accelerated sedimentation of the latter by means of dextran (M 500000) as described by Masson et al (21 23) with a few modifications After centrifugation at 500 g the leukocyte pellet is resuspended in 0 1 M phosphate buffer +1 0 M sodium chloride pH 8 0 The cells are disrupted by freezing and thawing 6 times (−20°C +37°C) and LF is determined in the supernatant after centrifugation at 750 g The PMN are counted in the leukocyte concentrate in order to calculate the LF concentration per 10⁶ PMN

Immunohistochemical techniques

Indirect immunofluorescent staining Citrate anticoagulated buffy coat smears are dried and fixed by means of absolute methanol for 3 min followed by incubation for 30 min with LF antiserum (absorbed with erythrocytes of the same person and rabbit liver powder dilution 1 240) and fluorescein isothiocyanate labeled goat antirabbit Ig (dilution 1 150) All steps were carried out at room temperature Following each stage smears were washed for 30 min in phosphate buffered saline pH 7 4

Immunoperoxidase staining direct method (25) EDTA anticoagulated buffy coat smears are dried and fixed for 30 sec by means of buffered formol acetone (20) After fixation slides are processed as follows Blocking of the endogenous peroxidase in absolute methanol containing 0 3% hydrogen peroxidase for 1 hour Incubation with peroxidase conjugated LF antiserum for 1 hour Development of the brown colour with DAB=3 3 diaminobenzidinetetrahydrochloride (0 6 mg/ml phosphate buffered saline pH 7 4) and hydrogen peroxide (0 01 %) for 8 min (10) Counterstaining with hematoxylin Following each stage smears were washed for 30 min in phosphate buffered saline pH 7 4

Iron determination

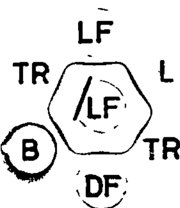
The plasma iron concentration and total iron binding capacity (TIBC) were estimated with the iron test combination of Boehringer Mannheim West-Germany

Statistical methods

Differences in LF concentrations numbers of leukocytes and iron concentrations were tested for statistical significance by Student s *t* test (7)

PATIENTS

Six groups of patients were studied The laboratory data are summarized in Table I



g 1 Immunodiffusion in agar *LF*=purified human ctoferrin *L*=leukocyte rich extract *TR*=tears *DF*= uodenal fluid *B*=bile */LF*=rabbit antiserum against uman LF The precipitation lines show immunological entity

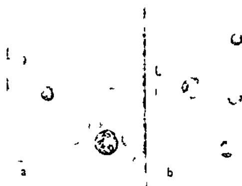


Fig 3 Direct immunoperoxidase staining of PMN *(a)* Diffuse cytoplasmic staining of PMN which is absent in mononuclear cells *(b)* After absorption of the LF antiserum with purified LF specific cytoplasmic staining of PMN is absent but non specific staining of eosinophilic leukocytes remains

RESULTS

ualitative determinations

F was identified in extracts of leukocyte rich amples by means of gel immunodiffusion (29) omplete fusion was observed of the precipitation nes of the leukocyte extract with LF of tears bile uodenal fluid and purified LF isolated from hu nan milk (Fig 1)

mmunohistochemical studies

ndirect immunofluorescence of PMN showed a right cytoplasmic staining (Fig 2) After absorp ion of the antiserum with purified LF the luorescence of PMN was abolished We did not ind a nuclear localization of LF as described by Green et al (11) Erythrocytes monocytes and ymphocytes showed no fluorescence whereas



Fig 2 Cytoplasmic fluorescence of PMN No nuclear localization of LF Indirect immunofluorescent technique

eosinophilic leukocytes retained their auto-fluorescence even after absorption of the LF anti serum with LF

With a direct immunoperoxidase technique all PMN showed diffuse cytoplasmic staining being absent in monocytes lymphocytes and thrombocytes Erythrocytes stained faintly on account of their high endogenous peroxidase activity (Fig 3) Eosinophilic leukocytes showed some non specific staining remaining after absorption of the LF antiserum with purified LF It is however very easy to distinguish eosinophilic leukocytes and PMN by the nuclei and the different cytoplasmic colour

Quantitative determination

LF was measured in the leukocyte concentrate by the single diffusion test (19) The LF concentration ($\mu g/10^6$ PMN) was calculated from

$$\frac{\text{amount of LF/ml leukocyte concentrate}}{\text{number of PMN/ml leukocyte concentrate}}$$

The control group (30 men and 6 women) had a mean LF concentration±S D of 7 7±1 9 $\mu g/10^6$ PMN There were no statistically significant differ ences between the sexes

Two of the control leukocyte concentrate sam ples were divided into 5 portions of 1 ml in each of which the LF concentrations and the numbers of PMN were measured separately The mean LF concentrations of the two samples were 5 4 and 7 4

Table II *LF concentration of PMN in patients and controls*

	LF concentration (µg/10⁶ PMN)			
Patient group	Mean	S E M	S D	Range
Controls	7.7	0.3	1.9	5.5–13.2
Iron deficiency anemia	6.3	0.2	0.4	5.8–6.9
Hemochromatosis	6.8	0.8	1.4	5.8–9.0
Hepatic cirrhosis	7.3	0.5	1.2	5.8–9.0
Acute hepatitis	7.7	1.7	2.4	5.4–11.0
Acute inflammation	4.8	0.5	1.3	3.0–7.6

µg/10⁶ PMN respectively In both cases the S D of the determination appeared to be very small (0.2) In 8 control leukocyte extracts LF was measured before and after storage at −20°C for one month LF concentrations after storage did not differ significantly from the fresh samples ($t=1.5$ $p>0.1$)

Repeated estimation of LF concentrations in PMN of one and the same patient at weekly intervals showed that LF concentrations fluctuate within narrow limits (range 6.2–6.9 µg/10⁶ PMN mean 6.5±0.2)

LF concentrations of PMN were studied in 5 groups of patients The hematological data are summarized in Table I The results were compared with the normal values These data are presented in le II and Fig 4

The mean LF concentration of PMN is significantly lower in patients with acute inflammation than in controls ($t=5.3$ $p<0.001$) although some overlap exists This difference proved to be related to the number of PMN per ml blood as we found a striking negative correlation between the number of PMN and the LF concentration of PMN ($r=-0.53$ $p<0.001$) As the total number of PMN per ml blood is significantly increased in these patients total LF in all PMN of 1 ml blood is the same as in normal controls or even higher

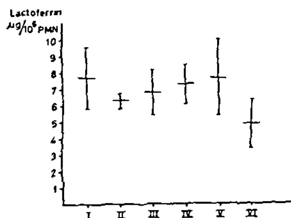


Fig 4 LF concentration of PMN (mean±S D) *I*=controls (n=36) *II*=patients with iron deficiency anemia (n=4) *III*=patients with hemochromatosis (n=4) *IV*=patients with hepatic cirrhosis (n=7) *V*=patients with acute hepatitis (n=3) *VI*=patients with granulocytosis as a result of acute inflammation (n=9)

Talbe III *Effect of blood donation on the LF concentration of PMN*

	LF concentration (µg/10⁶ PMN)	
Donor no	Before blood donation	10 days after blood donation
1	8.4	5.3
2	7.8	5.0
3	8.3	5.5
4	10.1	10.4
5	7.8	7.6
6	7.0	2.8
7	7.6	6.4
8	10.2	6.7
9	8.8	7.0
10	6.4	6.6
11	9.6	9.0
12	13.3	9.0
13	9.3	8.2
Mean	8.8	6.9
S E M	0.5	0.6
S D	1.7	1.9

As inflammations are often accompanied by disturbances of iron metabolism we studied in more detail the relation between the LF concentration of PMN and iron metabolism in controls in patients with iron deficiency and iron overload and in patients with liver diseases (acute hepatitis and hepatic cirrhosis) in whom also alterations of iron metabolism are frequently found Although none of these groups displayed a direct relation between the LF concentration of PMN and parameters of iron metabolism (plasma iron TIBC and Fe saturation) it is of interest that the mean LF concentration was significantly lower in patients with iron deficiency anemia (without granulocytosis) than in controls ($t=3.8$ $p<0.001$) The LF concentrations of PMN in patients with iron overload and liver diseases showed no significant differences from the controls although plasma iron was very high especially in acute hepatitis

In order to investigate the effect of iron loss or

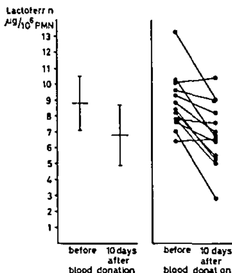


Fig. 5. LF concentration of PMN of 13 donors before and 10 days after blood donation. Mean values ±S.D. to the left, individual values to the right. There is a highly significant difference between the mean LF concentrations on the two days ($p<0.005$).

iron therapy on the LF concentration of PMN, we studied the following two groups of patients.

In 13 blood donors LF concentrations of PMN were measured just before and 10 days after blood donation. The results are presented in Table III and Fig. 5. The concentrations decreased in 10 of the 13 donors. The difference between the mean LF con-

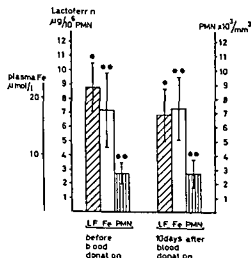


Fig. 6. LF concentration, plasma iron concentration and number of PMN of 13 donors before and 10 days after blood donation (mean±S.D.). * Statistically significant difference ($p<0.005$). ** not significant.

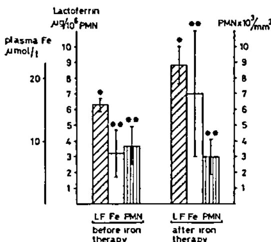


Fig. 7. LF concentration, plasma iron concentration and number of PMN of 4 patients with iron deficiency anemia before and after iron therapy (mean±S.D.). * Statistically significant difference ($p=0.01$). ** not significant.

centrations on these two days is highly significant ($t=4.29$, $p<0.005$). Plasma iron concentrations and numbers of PMN showed no differences (Fig. 6). In 4 donors LF concentrations of PMN were also measured on the 4th and 7th day after blood donation. The lowest level was mostly reached on the 4th day after donation, increasing afterwards.

In 4 patients with iron deficiency anemia the effect of iron therapy on LF concentrations, plasma iron and numbers of PMN was studied. The results are presented in Fig. 7. LF concentrations of PMN increased significantly in all 4 ($t=3.4$, $p=0.01$). As expected, the plasma iron concentrations also increased in all patients. The mean number of PMN showed no significant alterations.

DISCUSSION

In accordance with Masson et al. (21, 23) and Mason et al. (20) LF could be detected in the cytoplasm of mature PMN and bands using immunofluorescent and immunoperoxidase techniques. Masson et al. (23) showed that LF appears during the promyelocytic stage and that cytoplasmic concentration increases until the stage of mature PMN. The specific localization of LF in the nuclei of PMN, as reported by Green et al. (11), can be ascribed to an artifact due to fixation techniques in which ethanol has been used instead of methanol.

Only a few investigators measured LF in PMN

quantitatively The results were as follows Masson et al (21 23) 3 4±0 3 μg/10⁶ PMN Rumke et al (30) 5 0 Leffell and Spitznagel (16) 6 4±1 8 Olofsson et al (26) 4 4±1 2 Hansen et al (13) 1 82 μg/10⁶ PMN We found a higher value (7 7± 1 9 μg/10⁶ PMN) in our control group The reason for this is not clear but can be due to slight differences in technique

As shown by Hansen et al (13) low plasma LF concentrations correlate significantly with the total number of PMN in the peripheral blood and with the turnover rate of PMN which indicates that PMN are the main source of plasma LF Recently Hansen et al (12) studied LF concentrations of PMN and plasma during acute inflammation and found that the concentrations during the acute inflammatory phase were lower than in the convalescent stage Plasma LF concentrations during the acute phase, however amounted to 3 times the normal values In accordance with these observations we found a significantly lower LF concentration of PMN during the acute phase of inflammation The low LF concentration of PMN during the granulocytosis of acute inflammation might be explained by leakage of LF to the plasma On the other hand LF in the PMN often remains very low when the infection subsides and plasma LF is normalized again (12) It is also quite possible that LF synthesis in the bone marrow is decreased o data are available on this problem

he second part of our investigation concerned relation between LF of PMN and iron tabolism Although in individual patients no correlation was found between the LF concentration of PMN and parameters of iron metabolism loss of iron (donation of 450 ml blood) proved to be associated with a significant decrease in LF concentrations of PMN whereas iron therapy in patients with iron deficiency anemia resulted in significantly increased concentrations

From our results it may be concluded that LF in PMN depends on iron metabolism Iron deficiency seems to be accompanied by low LF concentrations and iron therapy augments LF concentrations of PMN The lowest LF concentrations of PMN were observed in acute inflammation possibly as a combination of iron deficiency and granulocytosis

These findings need not conflict with the theory of Bennett et al (3) van Snick et al (31) and Hansen et al (12) suggesting that during inflammation iron sequestration in the RES occurs due to macrophages taking up a LF-Fe complex in which the iron is derived from transferrin LF has a stronger binding capacity to iron especially with lower pH as occurs in the inflammatory area

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Long-term Effect of Clofibrate on Albumin Turnover and Distribution in Man

H Briem T Helgason and T V Gudmundsson

From the Departments of Clinical Biochemistry and Medicine Landspitalinn Reykjavik Iceland

ABSTRACT The long term effect of clofibrate (at least 6 months' treatment) on albumin metabolism was investigated in 7 subjects and the results were compared with those from 15 control subjects Human albumin labelled with ^{131}I was used as a tracer A significant difference between the groups was found in the following parameters The clofibrate treated group had a prolonged rapid component ($t_1\frac{1}{2}$) of the disappearance curve ($p<0.05$) relatively increased albumin in the extravascular space (i e decreased distribution ratio, $p<0.01$) and increased extravascular albumin space when corrected for body size by calculating it as per cent of the extravascular bromide space ($p<0.01$) There was no significant difference between the groups in albumin synthesis fractional catabolic rate or the slow component ($t_2\frac{1}{2}$) of the disappearance curve The results suggest that long term treatment with clofibrate causes changes in the intercellular matrix

Hard waxy exudates formed in diabetic retinopathy are believed to be primarily due to extravascular exudation of serum proteins and lipids (2 17) Clofibrate consumed for at least 6 months causes a marked reduction in these exudates (3 4) but this effect has not been explained Assuming that clofibrate might similarly influence other organs the effect of the drug in the diabetic nephrotic syndrome is presently being investigated Preliminary results indicate that clofibrate may beneficially affect the serum albumin level in this condition (5)

To test the effect of clofibrate on albumin metabolism an experiment was designed where the turnover and distribution of injected radioactive human albumin were studied in subjects receiving clofibrate for at least 6 months and the results were compared with those from a control group

STUDY POPULATION AND METHODS

Nineteen subjects were divided into two groups depending on whether or not they were receiving clofibrate Age height weight and some other relevant data are shown in Table I

Group 1 (clofibrate treated) consisted of 3 men and 4 women who had been receiving 1–2 g of clofibrate daily for at least 6 months Four subjects (nos 10 18 20 24) had ischemic heart disease two (nos 12 17) well controled diabetes without proteinuria and one (no 25) had slight hypercholesterolaemia All were living normal active lives but attending the Outpatient Clinic No side effect of the drug was observed in any of the subjects

Group 2 (controls) consisted of 9 males and 6 females who had not been receiving clofibrate for at least 6 months prior to the study Twelve 5 females and 7 males were healthy volunteers one (no 10) had ischemic heart disease and two (nos 12 17) had well controlled diabetes The latter 3 subjects were also tested while receiving clofibrate and are included in group 1

Serum cholesterol and triglyceride were estimated at the beginning of the study and the values are shown in Table I In addition the following laboratory tests were carried out at least twice during the study Hb haematocrit S ASAT S-LDH bilirubin blood urea urinary proteins and creatinine clearance in most cases All the results were within the normal range except that in subject 17 the Hb was between 110 and 120 g/l As there were no significant changes in any of these parameters or in body weight during the study period all the subjects were considered to be in a metabolic steady state

Clofibrate used in this study was supplied by Asgeir Sigurdsson Ltd the ICI agent in Iceland

Procedure

The experiment was carried out during five periods and therefore five different batches of labelled albumin termed A B C D and S were used Precipitation of the labelled albumin by adding an equal volume of 20% w/v trichloracetic acid to the labelled albumin solution showed that 2–4% of the radioactivity did not precipitate and was therefore considered to be due to free ^{131}I Each subject received intravenously a known dose of about 50 μCi ^{131}I labelled human albumin (The Radiochemical Centre Ltd Amersham England) Blood samples were

Table I *Clinical and laboratory data on the treated (group 1) and untreated (group 2) subjects*

Batch	Case no	Sex	Age (y)	Height (cm)	Weight (kg)	S-protein (g/l)	S-albumin (g/l)	Plasma volume (l)	Hb (g/l)
Group 1									
D	10	♂	48	180	95	75	54	3 21	140
D	12	♀	19	164	54	78	55	2 09	140
A	17	♀	54	167	74	70	41	2 42	110
B	18	♂	74	173	81	73	46	2 96	140
B	20	♀	59	157	59	70	47	1 90	160
S	24	♂	47	164	73	69	42	2 77	150
S	25	♀	30	164	60	76	42	1 87	130
Mean			47	167	71	73	47	2 46	139
S D			18 2	7 4	14 4	3 5	5 8	0 530	15 7
Group 2									
A	4	♂	26	185	88	80	46	3 15	140
A	5	♂	26	178	73	74	55	2 87	130
A	7	♂	67	164	71	76	43	2 59	160
B	9	♂	50	180	96	71	42	2 74	160
B	10	♂	48	160	95	74	46	3 18	160
B	11	♀	43	168	63	74	46	2 21	140
B	12	♀	19	164	54	73	46	2 13	140
C	13	♀	42	163	70	70	48	2 46	140
C	14	♂	29	181	85	74	49	2 66	150
D	17	♀	54	167	74	69	46	2 47	120
D	1	♂	43	170	70	72	50	2 31	150
[illegible]	2	♀	54	158	54	72	45	2 24	140
	3	♂	26	176	75	75	55	2 74	150
	8	♂	29	182	90	75	51	3 40	170
	23	♀	43	169	64	73	43	2.55	130
			40	172	75	73	47	2 65	145
S D			13 6	8 4	13 5	2 6	4 0	0 380	13 6
t test *p* value			>0 3	>0 2	>0 5	–	–	>0 3	>0 3

drawn for the determination of serum radioactivity and albumin concentration 15 min after the injection and thereafter daily for the next 5–7 days and then every 2nd–4th day throughout the study which lasted for 23–32 days Total protein was determined by the specific gravity method and serum albumin by the biuret method (8) The radioactivity of serum samples was counted in a well type scintillation counter The radioactivity of the samples was compared with a standard prepared from a known fraction of the preparation injected

Thyroid uptake was depressed by giving each subject 250 mg of potassium iodide in divided doses before the

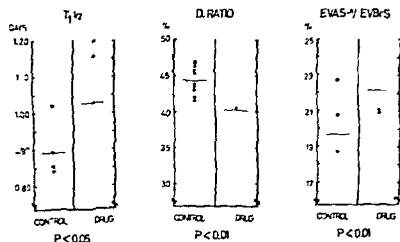


Fig 1 $T_{1/2}$ distribution ratio (*D RATIO*) and extravascular albumin as percentage of extravascular bromide space (*EVAS % EVBrS*) in the control and drug groups The horizontal lines indicate mean values

Hct (%)	S-cholesterol (mmol/l)	S-triglycerides (mmol/l)
42	5 9	0 78
42	4 8	0 69
32	5 0	1 36
44	5 6	0 83
46	8 1	1 52
43	6 0	1 21
41	8 9	2 12
41	6 3	1 23
4 5	1 56	0 495
44	7 2	0 62
41	5 1	0 34
48	7 3	0 77
46	6 7	2 11
46	9 5	1 02
43	7 8	0 74
43	5 6	1 01
41	4 9	0 98
43	5 5	0 99
36		
44	6 6	0 73
44	8 5	0 98
45	5 0	1 28
49	4 3	1 19
41	6 2	0 71
44	6 4	0 96
3 2	1 50	0 410
>0 05	>0 8	>0 2

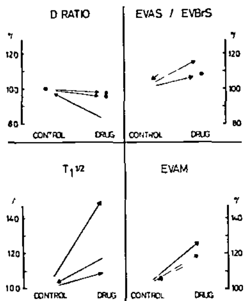


Fig 2 Changes in distribution ratio (*D RATIO*) extravascular albumin space as percentage of the extravascular bromide space (*EVAS %-EVBrS*) $t_1\frac{1}{2}$ and extravascular albumin mass (*EVAM*) in the three subjects tested twice The results are calculated as percental changes from the baseline when the subjects were off drug Two subjects were tested before taking the drug and then again after having received the drug for 6 months the third was first tested while on clofibrate and again 6 months after discontinuation of the treatment Arrows indicate sequence in time

injection of labelled albumin and thereafter 125 mg daily As none of the subjects had any indication of a renal disease non protein bound radioactive iodide in serum was disregarded in view of the high renal clearance of iodide The 24-hour bromide space was estimated in the majority of the subjects For this purpose each subject received 10 μCi ^{82}Br (The Radiochemical Centre Ltd Amersham England) in a 10 ml dose by mouth Urine was collected during 24 hours for correction for bromide loss As the bromide space was estimated 1–3 weeks after initiation of the albumin study the ^{82}Br was counted in a channel above the 722 kW peak of ^{131}I The counting of ^{131}I was delayed until the activity of ^{82}Br was insignificant compared with that of ^{131}I

Calculation

The metabolic parameters of albumin were calculated according to the method of Nosslin as cited by Andersen (1) The serum radioactivity was calculated as a fraction of the activity of the first sample drawn 15 min after the injection The serum activity curves for each subject were drawn on semilogarithmic graph paper independently by three individuals who did not know to which subject each curve belonged The curves were then analyzed by the peeling off technique (12) and the results presented are the means of the three curves drawn After the first 24 hours the radioactivity curves can be represented by a two-exponential function

$$P=c_1e^{-b_1t}+c_2e^{-b_2t}$$

Zero time is the time 15 min after the injection of the radioactive albumin b_2 the slope constant of the latter exponential function ($=0\ 693/t_2\frac{1}{2}$) c_2 the intercept with the ordinate at zero time expressed as a percentage of the activity of zero time b_1 and c_1 represent the same parameters for the first exponential function To obtain a reliable final slope of the plasma curve the radioactivity in plasma was followed for 23–32 days

The following calculations were used *Plasma volume* (100×0 98)/(% dose/l plasma in the 15-min sample) Factor 0 98 is based on the assumption that 2% of the injected dose has left the intravascular space after the first 15 min *Intravascular albumin mass (IVM)* Plasma volume×albumin concentration (the mean concentration of albumin during the sampling period) *Total exchangeable albumin mass (TM)* $IVM\times\Sigma c/b^2/(\Sigma c/b)^2$ *Extravascular albumin mass* TM−IVM *Fractional catabolic rate*

Table II *Results of the ¹³¹I labelled study*

Group 1=treated with clofibrate, group 2=controls
EVAS=extravascular albumin space EVBrS=extravascular bromide space

Case no	Intra vascular albumin mass (g)	Extra vascular albumin mass (g)	Distribution ratio (%)	Rate of albumin synthesis (g/d)	Fractional catabolic rate (%)	Half time of the first exponential slope ($t_{1/2}$) (d)
Group 1						
10	173	290	37 4	24 6	14 2	0 85
12	115	167	40 7	15 5	13 5	1 19
17	99	187	34 6	11 0	11 2	1 16
18	136	177	43 4	13 4	9 8	0 98
20	89	120	42 5	12 3	13 8	1 03
24	116	161	41 9	14 1	12 2	0 81
25	79	107	42 5	9 7	12 3	1 20
Mean	115	173	40 4	14 4	12 4	1 03
S D	31 7	59 5	3 24	4 91	1 56	0 161
Group 2						
4	145	187	43 7	15 1	10 4	0 95
5	158	175	47 5	16 4	10 4	0 83
7	111	148	42 9	11 8	10 6	0 94
9	115	175	39 6	16 4	14 3	0 75
10	146	240	38 0	19 0	13 0	0 79
11	102	110	48 2	9 4	9 2	1 17
12	98	131	42 8	12 2	12 5	0 76
13	118	134	46 8	16 1	13 7	0 85
14	130	151	46 2	17 0	13 1	0 89
	114	158	42 0	12 9	11 3	0 94
	116	143	44 9	14 8	12 7	0 91
	101	121	45 6	11 7	11 6	0 84
	151	177	46 1	14 9	9 9	0 87
8	173	199	46 5	19 0	11 0	0 83
23	110	132	45 4	9 2	8 3	1 02
Mean	126	159	44 4	14 4	11 5	0 89
S D	23 2	34 0	2 81	3 09	1 72	0 108
Student s *t* test *p* value	>0 3	>0 5	<0 01	–	>0 2	<0 05

$1/\Sigma c/b$ *Rate of albumin synthesis* (mass of albumin synthesized per day) IVM/∫Pdt or IVM/$\Sigma c/b$ *Distribution ratio (IVM/TM)* $(\Sigma c/b)^2/\Sigma c/b^2$ *Albumin space* TM/albumin concentration in serum (mean) *Apparent Br space* $(ads-vu)/1000 \times p$ (a is the volume of injected dose (ml) d the dilution factor of standard s the count rate of diluted standard u the count rate of urine sample/ml v the volume of collected urine during 24 hours and p the count rate of plasma sample drawn 24 hours after the dose is given) *Extravascular albumin space* Albumin space–plasma volume *Extravascular bromide space* 24 hours bromide space–plasma volume The results were compared by Student s t test

RESULTS

The main results, including 1 S D and the p values from Student s t test, are given in Table II and Fig 1 They show the following significant differences between the two groups

The distribution ratio is smaller in the clofibrate treated subjects (group 1) than in the controls (group 2) (group 1 40 4±3 24% group 2 44 4 ±2 81% $p<0 01$) the half time of the first exponential slope ($t_{1/2}$) is longer (group 1 1 03± 0 16 days group 2 0 89±0 11 days $p<0 05$) and the extravascular albumin space calculated as a percentage of the bromide space is greater in the treated than in the control group (group 1 22 1±1 54% group 2 19 6±1 49% $p<0 01$) (Fig 1)

The drug did not cause any significant change in the rate of albumin synthesis (group 1 14 4±4 91

Half time of the second exponential slope ($t_{1/2}$) (d)	EVAS (l)	EVBrS (l)	EVAS (% of EVBrS)
13 8	5 37	22 1	24 3
13 7	3 04	13 2	23 0
19 1	4 56	19 3	23 6
17 3	3 85	18 3	21 0
12 7	2 55	12 6	20 3
14 3	3 83	17 8	21 5
14 1	2 55	12 2	20 9
15 0	3 68	16 5	22 1
2 30	1 052	3 85	1 54
16 1	4 07		
14 8	3 18		
16 2	3 44		
12 9	4 17	20 4	20 4
15 1	5 21	23 1	22 6
16 7	2 39	13 9	17 2
13 5	2 85	14 3	19 9
11 6	2 79		
17 1	3 08		
15 9	3 43	18 6	18 4
12 8	2 86	15 2	18 8
13 9	2 69	13 5	19 9
16 3	3 22	16 4	19 6
14 4	3 90	21 0	18 6
19 0	3 07	14 8	20 7
14 8	3 36	17 1	19 6
2 00	0 723	3 41	1 49
>0 8	>0 4	>0 7	<0 01

g/d group 2 14 4±3 09 g/d) the fractional catabolic rate (group 1 12 4±1 56%/d group 2 11 5 ±1 72%/d $p>0$ 2) or the half time of the second expontential slope ($t_{1/2}$) (group 1 15 0±2 30 days group 2 14 8±2 00 days $p>0$ 8)

Subjects 10 and 12 were tested before and after receiving clofibrate When taking clofibrate the distribution ratio was lowered the $t_{1/2}$ was prolonged and the ratio between extravascular albumin space and bromide space was increased However the reverse occurred in one subject (no 17) in whom the first test was performed while on clofibrate and the second test after 6 months off clofibrate (Fig 2) When these three individuals were receiving clofibrate there was a notable increase in the extravascular albumin mass (Fig 2) but the mean values of this parameter for the two groups do not differ significantly

DISCUSSION

The method described in this paper for studying the albumin metabolism is by now a standard procedure and the results presented are in the same range as those published earlier (16)

The lack of effect of the clofibrate treatment on the rate of albumin synthesis fractional catabolic rate and half time of the second exponential slope ($t_{1/2}$) makes it unlikely that clofibrate has a direct effect on albumin metabolism This study does not indicate whether clofibrate might beneficially effect the serum albumin levels in diabetics with heavy proteinuria There is an increase in $t_{1/2}$ in the treated group which presumably means a slower net outflow of albumin from the vascular space This does not cause an increase in plasma albumin in normals and the increase cannot be explained from the present data

The observed differences of a smaller distribution ratio and a larger extravascular albumin space without any change in extracellular water space during clofibrate treatment can be explained either by adsorption of albumin on the intercellular matrix or a true increase in the albumin space but the present data do not distinguish between these two possibilities

It is known that the intercellular matrix consists of high molecular weight proteoglycans (11) The three-dimensional structure of these macromolecules is thought to form a network which restricts the volume into which other large molecules may diffuse i e extravascular albumin is confined to only a part of the intercellular space (11 13 15) The results presented may therefore be explained by clofibrate influencing the intercellular matrix in such a way that a greater space would be available for albumin to diffuse into that is to increase the extravascular albumin space in relation to the unchanged extravascular extracellular water space this would cause a decrease in the distribution ratio

This supposed effect of clofibrate on the intercellular matrix making more space available for macromolecules to diffuse into would lead to a fall in the plasma concentration of those molecules However large molecules under homeostatic con

trol such as albumin (14 15) would be expected to have the same plasma concentration but increased total mass There is a difference in the extravascular albumin mass between the groups showing a trend in that direction although not statistically significant Furthermore in all the three cases tested twice the extravascular albumin mass was increased during clofibrate treatment

Exudates formed in diabetic retinopathy are believed to be deposits of serum proteins and lipids (2 17) These exudates are reduced by long term treatment with clofibrate the drug however does not have any demonstrable effect on vascular changes in diabetic retinopathy (3 4) It has been shown repeatedly that macromolecules like high molecular weight dextrans and hyaluronic acid which form the extracellular matrix decrease the solubility of other macromolecules by steric exclusion (6 7 9 10) In diabetic retinopathy the damaged vessels allow exudation of plasma components under pressure into the extravascular space Therefore it may be expected if the exudation is large enough that the steric exclusion of the matrix will lead to the precipitation of plasma macromolecules The suggested effect of clofibrate to decrease the exclusion of the interstitial matrix on romolecules would diminish the possibility of ipitation of the macromolecules

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Effects of Glipizide and Food Intake on the Blood Levels of Glucose and Insulin in Diabetic Patients

G Sartor B Schersten and A Melander

From the Departments of Internal Medicine and Clinical Pharmacology University of Lund University Hospital Lund Sweden

ABSTRACT The blood levels of glucose and insulin were monitored in 15 patients with non ketotic maturity onset diabetes mellitus given a single 5 mg oral dose of a novel sulfonylurea drug, glipizide, together with and 30 min prior to, a standardized breakfast meal In addition, the effect of the meal only was examined The mean preprandial levels of blood glucose and plasma insulin on the three occasions were similar Following glipizide administration the early postprandial increment of blood glucose was reduced, and there was a subsequent decrease to values below the preprandial level Both the decrease and the reduction of the early increase were significantly more marked when the drug was given 30 min prior to than together with the meal Apparently, this did not result from a stronger effect of the drug on insulin release when given before than together with the meal, as the plasma insulin levels and the corresponding concentration curve areas did not differ Rather, the difference was due to a more optimal timing between insulin release and the blood glucose increment evoked by the meal In addition there seemed to be only a minor interindividual variation in the bioavailability of the drug as its effect on insulin levels varied rather little Hence, 5 mg of glipizide three times daily 30 min before meals can be recommended as a standard regimen

Sulfonylurea drugs are frequently used in the treatment of patients with diabetes of the maturity-onset type The rationale for this therapy is that such drugs are capable of reducing the blood glucose levels and there is evidence that this effect at least partially results from a drug induced release of insulin from the beta cells of the pancreatic islets (3 8) The currently used sulfonylurea drugs are assumed to have a similar or identical mechanism of action but they differ in potency and in onset and duration of action (3 5 8 9)

Normally the glucose and insulin levels in blood fluctuate greatly and it is reasonable to assume that the best therapeutic effect is obtained with the drug regimen that most closely mimics the normal pattern of insulin secretion In particular it is important to explore the most appropriate interval between food ingestion and drug administration as the blood levels of glucose and insulin vary in response to meals

The present study concerns the effects of a new sulfonylurea drug glipizide (1) on the blood levels of glucose and insulin in diabetic patients given the drug together with and 30 min prior to a standardized breakfast meal

STUDY POPULATION AND METHODS

Eleven male and four female subjects aged 27–70 (mean 56) volunteered for the study All had non ketotic maturity-onset diabetes mellitus of moderate degree and a duration of 0 5–4 years Their body weight range was 60–100 kg (mean 79) Prior to the study none of them had received any oral antidiabetic drugs or insulin and none had been given thiazides or corticosteroids No complicating disease was present

Each subject came in a fasting state to the Out Patient Clinic at 8 a m on three different occasions at intervals of 5–10 days to be studied with respect to the effect of *a*) a standardized meal *b*) glipizide together with the meal and *c*) glipizide 30 min before the meal Glipizide was given as single 5 mg tablets all of the same brand and batch (Mindiab® Carlo Elba Milano Italy) In order to avoid effect variations due to changes in the dietary regimens during the test period 7 patients were studied in the order *c–a–b* and 8 in the reverse order i e *b–a–c*

trol such as albumin (14 15) would be expected to have the same plasma concentration but increased total mass There is a difference in the extravascular albumin mass between the groups showing a trend in that direction although not statistically significant Furthermore in all the three cases tested twice the extravascular albumin mass was increased during clofibrate treatment

Exudates formed in diabetic retinopathy are believed to be deposits of serum proteins and lipids (2 17) These exudates are reduced by long term treatment with clofibrate the drug however does not have any demonstrable effect on vascular changes in diabetic retinopathy (3 4) It has been shown repeatedly that macromolecules like high molecular weight dextrans and hyaluronic acid which form the extracellular matrix decrease the solubility of other macromolecules by steric exclusion (6 7 9 10) In diabetic retinopathy the damaged vessels allow exudation of plasma components under pressure into the extravascular space Therefore it may be expected if the exudation is large enough that the steric exclusion of the matrix will lead to the precipitation of plasma macromolecules The suggested effect of clofibrate to decrease the steric exclusion of the interstitial matrix on macromolecules would diminish the possibility of ipitation of the macromolecules

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60	90	120	150	180
10 1±0 4	9 3±0 5	8 6+0 5	7 4±0 8	7 1+0 7
9 2±0 6	7 5±0 6 <0 05	6 6±0 6 <0 05	4 9±0 8 <0 05	4 5±0 7 <0 05
7 9±0 6 <0 01	6 3±0 5 <0 001	5 3±0 5 <0 001	3 8±0 8 <0 01	4 0±0 6 <0 01

(Fig 1 Table I) The respective mean postprandial blood glucose values were significantly lower at 90–180 min when glipizide was given with the meal ($p<0\ 05$) and already from 45 min when the drug was given 30 min before the meal ($p<0\ 05$–$0\ 001$) as compared to the meal alone

The mean preprandial plasma insulin levels were 18 16 and 17 μU/ml respectively Following the meal the mean plasma insulin level increased significantly but slowly and never exceeded 50 μU/ml (Fig 1 Table II) When glipizide was combined with the meal the mean plasma insulin level rose to significantly higher levels ($p<0\ 05$–$0\ 01$) compared to the meal alone reaching a maximum of about 80 μU/ml Similar maximum levels were seen when glipizide was given 30 min before the meal but the increment occurred more rapidly Indeed compared to the meal alone a significant elevation ($p<0\ 05$–$0\ 001$) of the plasma insulin concentration was recorded already at the start of the meal (Fig 1 Table II)

Table III presents the incremental and decremental areas derived from the blood glucose concentration curves It can be seen that the mean incremental area following the meal as such was 2 7 times larger than that resulting from administration of glipizide prior to the meal and 1 6 times larger than that resulting from the concomitant administration of glipizide and the meal The differences were significant at $p<0\ 001$ and $p<0\ 05$ respectively Table III further shows that the mean decremental area following glipizide ingestion 30 min before the meal was 12 times larger than that following the meal as such When glipizide was given together with the meal the mean decremental area was 5 times larger than that following the meal as such Both differences were significant at $p<0\ 05$

	90	120	150	180
)+ 7	49± 6	44± 5	29± 4	22+ 4
6±10 0 05	80±11 <0 05	83±11 <0 01	65±16 <0 05	48+13
¹± 8 0 05	80± 9 <0 01	78±13 <0 05	62±18	42±14

Table III *Incremental and decremental areas of blood glucose curves and areas of plasma insulin curves (arbitrary units) at the three types of treatment (mean±S E M)*

	Glipizide		
	Together with the meal	30 min before the meal	Meal only
Blood glucose			
Incremental areas	2 382±381	1 464±343	3 882±448
p	<0 05		
p^b		<0 001	
Decremental areas	725±260	1 568±5‿6	131±125
p^a	<0 05		
p^b		<0 05	
Plasma insulin			
Areas	5 810±792	6 239±739	3 038±370
p	<0 01		
p^b		<0 001	

Significance of the difference between the meal only and glipizide together with the meal [b]30 min before the meal

The mean areas of the insulin concentration curves resulting from administration of the drug prior to the meal and from the concomitant administration respectively were significantly larger than that resulting from the meal as such The differences were significant at $p<0\ 001$ and $p<0\ 01$ respectively (Table III)

DISCUSSION

The present findings demonstrate that glipizide is a potent and rapidly acting antidiabetic agent (1 7)

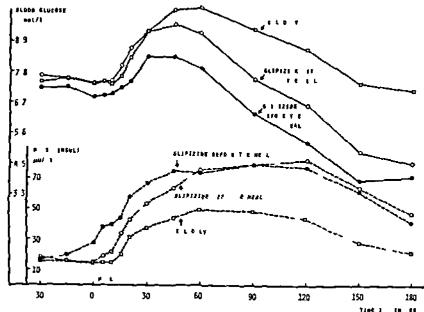


Fig 1 Mean levels of blood glucose and plasma insulin in diabetic patients given glipizide 30 min before and together with a breakfast meal

only 5 mg are needed to evoke significant increments and reductions respectively of the insulin and glucose levels in blood and such effects can recorded within 15–30 min after drug administra n

The most pronounced antidiabetic effect was ob ned when glipizide was administered 30 min before the meal as evidenced both by lower blood glucose levels and by smaller incremental and larger decremental areas of the blood glucose concentration curves Apparently this did not result from a stronger effect of the drug on insulin release when given before than together with the meal as the plasma insulin levels and the corresponding concentration curve areas did not differ Rather it was due to a more optimal timing between induction of insulin release and the blood glucose increment evoked by the meal

In addition to these observations it appears that the bioavailability of the drug—which is reported to be absorbed virtually completely (2 7)—is subject to only a minor interindividual variation as its effect on insulin levels varied relatively little Therefore 5 mg of glipizide three times daily 30 min before meals can be recommended as a standard regimen

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Neuropsychological Findings with Pseudoxanthoma Elasticum

R K Heaton A T Vogt K H Neldner and E B Reeve

From the Departments of Psychiatry Dermatology and Medicine University of Colorado Medical Center Denver Colorado USA

ABSTRACT It has been reported that the disease pseudoxanthoma elasticum (PXE) is associated with a high incidence of neurologic and psychiatric symptoms, which are possibly due to cerebrovascular ischemia These reports are based mainly upon clinical observations made without reference to control group data We have compared the results of 27 PXE patients with results of a control group on a battery of objective neuropsychological and personality tests The PXE group showed only very mild deficits on extensive neuropsychological testing, a finding which argues against any marked involvement of cerebral vessels in these patients Similarly, the personality test results did not reveal significant psychiatric disturbances which could be attributed to PXE Possible reasons for the discrepancy with previous reports include the subjective data and much older PXE patients on which those reports are based The need for systematic neuropsychological research with older PXE patients and control groups is suggested

Pseudoxanthoma elasticum (PXE) is a connective tissue disease of man characterized by aberrant calcification of the elastic tissue of the skin eyes and vasculature (16) The skin lesions are small slightly raised orange yellow papules appearing in the regions of the neck axillae cubital fossae periumbilical area groins and popliteal spaces These usually commence during early adolescence but sometimes earlier The ocular lesions consist of angioid streaks or cracks in the calcified Bruch s membrane which supports the retina Hemorrhage may occur through the angioid streaks and produce loss of central vision (17) Vascular lesions are the most dangerous appearing as degeneration and calcification of the elastic lamellae of the medium size arteries such as the radial coronary popliteal posterior tibial and perhaps the cerebral arteries (9) Partial or complete occlusion of arteries leads to loss of peripheral pulses intermittent claudication angina and other evidence of ischemia (9 16) Hemorrhage of gastric arteries may lead to severe gastrointestinal bleeding Hypertension is a common complication and is sometimes caused by sclerosis of a renal artery (7)

Although calcification and occlusion of peripheral arteries occur with sufficient frequency to infer a direct relationship with PXE the association between PXE and cerebrovascular disorders is not so clear There have been isolated reports of cerebrovascular accidents and calcification of certain cerebral structures usually in elderly PXE patients but such findings may be entirely coincidental to this disease Several investigators have reported an unusually high incidence of neurasthenia poor memory and various emotional disturbances with PXE and have speculated about a vascular origin of these symptoms (1 3 6 15) In one series of 29 PXE patients 26 were considered to have such mental symptoms which were often severe enough to significantly impair the patients abilities to work (3) These reports of disabling neurologic and psychiatric symptoms are based upon clinical observations and patient reports not upon objective psychological testing Furthermore no control group has been used to show that the incidence of mental disorders is abnormally high among patients having PXE

In the present study we compare the results of a PXE patient group with those of a control group on a battery of neuropsychological and personality tests

SUBJECTS

Twenty seven patients with PXE and 27 normal controls were studied Sixteen of the PXE and 14 control subjects were females The mean age of the PXE group was 31 9 years (S D 11 2) which is similar to the control group mean of 31 7 years (S D 11 8) The groups also had comparable educational backgrounds the means were 12.4 years (S D 2 6) for the PXE and 12.7 years (S D 2.5) for the control subjects No subject from either group had history of disease or significant trauma involving the brain

METHODS

Psychological testing Subjects were tested individually by trained and experienced neuropsychological technicians For each subject the testing was completed in a single working day usually in about seven hours

The tests consist of the Wechsler adult intelligence scale (WAIS 26) and an expanded version of the test battery originally developed by Halstead (10) and Reitan (19) Numerous studies published over the last 30 years have shown that these tests are sensitive to focal and diffuse cerebral lesions caused by diverse neurologic conditions including cerebrovascular disease (8 18 21 23) Recent reviews of this literature are available in Reitan and Davison (22) and Russell et al (24)

Our expanded Halstead Reitan battery includes meas of intelligence attention various cognitive functions proficiency sensory perceptual functions aphasia related disorders and verbal learning and memory An objective personality test has also been given in an effort to confirm clinical impressions that PXE patients show increased tendencies toward psychiatric disturbances The following are brief descriptions of the specific tests and test scores used in this study More detailed descriptions can be found in the references provided

Wechsler adult intelligence scale (26) The WAIS is a well known and widely used measure of adult intelligence (14) Scores used here are the verbal performance and full scale IQ values and the scaled scores on the individual subtests (information comprehension arithmetic similarities digit span vocabulary digit symbol picture completion block design picture arrangement and object assembly)

Halstead category test (10 20) This is a relatively complex nonverbal test of abstraction and concept formation The subject s goal in the first six subtests is to determine a unifying principle that when applied to each item on the subtest will give the correct answer A seventh subtest is a review group where the subject tries to remember the answer to items seen in the earlier subtests The score is the number of errors on the total of 208 items

Tactual performance test (10 20) In this test there are three trials in which the subject is blindfolded and asked to place 10 geometrically shaped blocks into their correct spaces on a form board. The first trial is done with the dominant hand trial two with the nondominant hand and on the third trial both hands are used The three trials are timed and a maximum of 10 min is allotted for each The measure we are using to reflect psychomotor problem solving efficiency is the time (min) taken per block for the three trials combined

There are also two measures of incidental memory generated on the tactual performance test The subject is not told in advance to remember anything about the blocks or the board However after the three trials are completed the board is removed the blindfold is taken off and the subject is asked to draw a picture of the form board from memory Memory points are earned for correctly recalling the shapes of the spaces on the board and "location" points for shapes correctly localized on the drawing. A maximum of 10 points is possible on each of the measures.

Speech sounds perception test (10 20) This test requires sustained attention accurate perception of verbal auditory stimuli and the ability to match simple spoken words with their written versions on an answer sheet. Sixty nonsense words are presented from a tape recorder each having a middle "ee" vowel sound and different consonant combinations at either end Each of the 60 spoken nonsense words must be selected (underlined) from among four written alternatives on the answer sheet. The score recorded is the number of errors made on the 60 items

Seashore rhythm test (10 20 25) This test requires sustained attention fine discrimination among nonverbal auditory stimuli plus short term memory for such stimuli. The subject is presented 30 pairs of rhythms via a tape recorder and for each pair is required to indicate whether the second rhythm is the same as the first or different. The score recorded is the number of correct judgments out of a possible 30

Finger oscillation test (10 20) This is a test of motor speed with the upper extremities It requires the subject to tap as fast as he can with his index finger using an apparatus which resembles a telegraph key The mean number of taps on five 10-second trials is recorded for each hand and in this study these two figures are summed to give a final measure of tapping speed.

Halstead impairment index (10 20) In current practice this summary measure of generalized neuropsychological deficit uses seven of the test scores described above: category tactual performance test total time memory and location Seashore rhythm speech-sounds perception and finger oscillation with the dominant hand only The index is the proportion of scores on these tests which are in the range characteristic of patients with documented cerebral lesions Higher index scores increase the probability of impaired cerebral functioning

Trail making test parts A and B (2 20) These are paper and pencil tests requiring general alertness spatial analysis motor speed and the ability to follow a correct sequence of numbers (in part A) or numbers and letters (in part B) Part B is more complex in that it requires the subject to follow letter and numerical sequences in alternating fashion The score recorded is the number of seconds taken to complete both parts

Aphasia screening examination (20 27) This is Reitan s modified version of the Halstead Wepman aphasia screening test (11) The test "screens" (rather than sampling the relevant abilities in much detail) for deficits in the subject s abilities to name common objects spell

simple words identify letters and numbers read and write simple words and short statements enunciate repeat a short statement and explain its meaning work simple math problems demonstrate the use of a common object (key) and discriminate right from left The scoring and item weighing system of Russell et al (24) was used to derive a total aphasia score on this test which can range from 0 to 75

Spatial relations (24) This is a measure of constructional dyspraxia or degree of spatial distortion apparent in the subject s reproductions of geometric designs The score used is the rating of Russell et al (24) based upon the subject s drawing of the Greek cross from the aphasia screening exam (27) and his scaled score on the WAIS block design subtest

Reitan Klove sensory perceptual examination (20) From this examination we administered the tests for finger tip number writing imperception (graphesthesia) tactile finger recognition errors (finger dysgnosia) and sensory suppressions (tactile auditory and visual extinction phenomena) For each test error scores on the two sides of the body are summed and these sums are added together to make a total perceptual error score

Average impairment rating (24) This score is the average of the 0 (better than average) to 5 (severely impaired) ratings which the subject earns on 11 of the Halstead Reitan battery tests described above and one WAIS measure It differs from the Halstead impairment index in that it includes more tests and in that it reflects degree of overall impairment rather than just range of abilities affected

Reitan Klove tactile form recognition (20) This is a test of stereognosis which requires the subject to discriminate among four flat plastic shapes by touch alone A vertically positioned board is used which has copies of the shapes mounted on the board There are eight trials with each hand The score used in this study is the time (sec) taken to complete the total 16 trials

Smedley hand dynamometer This test of grip strength is often included in the Halstead Reitan battery for clinical and research investigations (22) Two trials are given with each hand and the average strength for each recorded in kg The final score is the sum of these mean scores for the two hands

Klove Matthews motor steadiness battery (22) The grooved pegboard test and the hole type steadiness test are used in this study

The grooved pegboard (Lafayette Instrument Co no 32035) measures speed and fine motor coordination with the upper extremities It requires the subject to place 25 small metal pegs into holes on a horizontal board as quickly as possible The holes have grooves on one side so the pegs will not fit unless they are rotated properly A trial is given with each hand The number of seconds taken to place all 25 pegs are recorded and summed across the two trials

The hole type steadiness test (Lafayette Instrument Co no 32011) is a test of static steadiness The subject is asked to hold a stylus in the center of six successively smaller holes trying not to let the stylus touch the sides He is not allowed to support the hand or arm he is using for this on the table or with his body or other hand The stylus is connected to an electrical timer counter device which automatically records the number of contacts with the sides of the holes (errors) Both hands are tested for all six holes and error scores are summed across the 12 trials

Modified Reitan story memory test part A (original version is an unpublished test obtained from the author) This is a test of verbal learning and memory As in the unmodified version during the learning phase the subject is presented a short story over a tape recorder and then asked to repeat back as much of the story as he can If on this first trial he cannot repeat back a minimum of 15 of the 28 bits of information in the story he is given up to four more learning trials to reach this criterion Memory testing for the story is done four hours after the learning phase is completed these four hours are taken up by other testing and usually a lunch break Efficiency of learning is operationally defined as the number of learning trials (0–5) taken to reach criterion The memory score is the difference between the amount of information (bits) reported at the last learning trial and the amount of information recalled four hours later

Minnesota multiphasic personality inventory (MMPI 12) The MMPI provides objective measures of major dimensions of psychopathology and an extensive body of published research supports its validity for this purpose (4 5) The PXE and control groups were compared on the three MMPI validity scales and the following standard clinical scales hypochondriasis depression hysteria psychopathic deviate paranoia psychasthenia schizophrenia and mania

RESULTS

Mean neuropsychological test scores of the PXE group were compared with control group means using t tests after determining by F tests whether pooled or separate variance estimates were appropriate Table I presents the results of these comparisons

The PXE group scored significantly worse than the controls on the average impairment rating and the Halstead impairment index This indicates that the PXE patients scored in the impaired range on more of the seven Halstead measures and showed greater average impairment on the Halstead Reitan battery than did the controls However the PXE means on these summary measures of neuropsychological deficit are not in the range characteristic of patients with known cerebral lesions Furthermore although the PXE group means are somewhat worse than those of the control group on most of the individual test measures derived from the Halstead Reitan battery these differences are statistically significant only for the trail making test the tactual performance test (total time per block) and the Seashore rhythm test Similar findings were

Table I *Means S D and t test results of neuropsychological variables*

Data for sensory perceptual and motor tests are the sum of performances with the left right and both hands where applicable

	PXE		Control		
	Mean	S D	Mean	S D	*t*
Summary measures					
Average impairment rating	1 04	0 41	0 83	0 33	2 02*
Halstead impairment index	0 35	0 25	0 23	0 20	2 07*
Halstead Reitan battery tests					
Category (errors)	49 6	25 3	38 9	19 8	1 74
Trail making (A+B) (sec)	105 3	38 7	85 9	21 0	2 29*
Tactual performance					
Time/block (min)	0 5	0 2	0 4	0 2	2 04*
Memory (correct)	7 6	1 3	7 8	1 1	0 79
Location (correct)	5 5	2 3	4 5	1 7	1 83
Speech sounds perception (errors)	5 5	3 4	5 3	2 8	0 26
Seashore rhythm (correct)	25 5	2 6	27 9	1 6	4 11**
Finger oscillation (no /20 sec)	91 2	13 0	95 3	13 5	1 13
Tactile form recognition (sec)	20 2	5 5	17 9	3 1	1 83
Aphasia screening (errors)	3 8	3 7	3 0	3 1	0 93
Spatial relations (rating)	1 1	0 3	1 1	0 3	0 00
Sensory perceptual exam (errors)	4 3	3 4	3 2	2 9	1 26
Wechsler adult intelligence scale					
Intelligence quotients					
Full scale	106 6	13 0	111 1	10 7	1 41
Verbal	106 6	12 4	112 1	12 6	1 63
Performance	105 0	13 8	108 4	9 8	1 06
Scaled scores					
Information	11 0	2 5	11 5	2 5	0 71
Comprehension	12 3	3 1	12 6	3 1	0 44
Arithmetic	9 9	3 4	11 5	3 1	1 77
Similarities	11 0	2 4	12 7	2 7	2 49*
Digit span	10 3	2 5	11 3	2 9	1 42
Vocabulary	11 5	2 5	11 7	2 8	0 20
Digit symbol	10 7	2 2	11 8	3 2	1 45
Picture completion	9 8	2 7	10 4	1 8	0 90
Block design	10 3	2 3	10 8	2 0	0 81
Picture arrangement	9 9	2 6	10 5	2 5	0 95
Object assembly	10 7	2 8	10 1	2 0	0 81
Added tests					
Story memory–learning	1 8	0 6	1 6	0 8	1 09
Story memory–memory	2 2	2 4	1 6	2 3	0 92
Hand dynamometer (kg)	72 1	22 7	80 4	25 5	1 25
Grooved pegboard (sec)	144 3	25 4	134 1	15 1	1 80
Hole type steadiness (hits)	42 2	25 9	53 4	44 7	1 13

*$p<0\ 05$ **$p<0\ 01$ (two-tailed)

obtained with the WAIS and the added tests of motor functioning learning and memory on most measures the PXE group means are slightly worse than the control group means but only one com parison out of 19 yields a statistically significant difference

Table II presents group comparisons on personal ity measures assessed by the MMPI None of the validity scales and only one of the eight clinical scales are significantly different between the groups, with the PXE patients scoring higher on the hypochondriasis scale

Table III shows ordinal correlations between the PXE patients neuropsychological summary scores and independent ratings of physical symptoms as sociated with PXE

The skin rating is the total area of skin which showed visible PXE lesions The eye involvement measure is the number of angioid streaks The scale of peripheral arterial involvement is based upon limb blood pressure gradient measurements at rest and during exercise and in some cases measure ments of pulse wave velocity contours at the femoral artery determined by Doppler methods

Table II *Means S D and t test results of personality variables (T scores)*

	PXE		Control		
	Mean	S D	Mean	S D	*t*
MMPI					
Validity scales					
Lie	46 3	5 9	48 2	5 6	1 22
Validity	56 1	10 9	53 7	8 5	0 91
Defensiveness	55.3	8 8	55 1	10 1	0 08
Clinical scales					
Hypochondriasis	58 3	11 2	51 7	8 0	2 46*
Depression	58 0	12 5	53 1	9 2	1 64
Hysteria	59 9	9 7	57 5	6.3	1 07
Psychopathic deviate	60 5	12 2	58 3	11 2	0 66
Paranoia	55 4	9 5	56 6	8 3	0 49
Psychasthenia	57 5	10 0	56 4	9 9	0 40
Schizophrenia	60 1	13 3	54 3	9 4	1 82
Mania	56 2	8 0	57 7	10 8	0.59

* $p<0\ 05$ (two-tailed)

None of the correlations listed in Table III are statistically significant suggesting that the degree of neuropsychological deficit is independent of degree of skin eye and peripheral arterial involvement in these patients It is also of interest that of the six correlations among the physical symptom ratings themselves only that between number of angioid streaks and peripheral arterial involvement is statistically significant ($\tau=0\ 35$ $p<0\ 05$)

DISCUSSION

Any impairment of brain functions which is associated with PXE would most likely be due to patchy vascular lesions Therefore interpretation of our findings relies upon the sensitivity of our tests to diffuse cerebrovascular disease Reitan (21) administered most of these tests to a normal control group a group of patients with diffuse cerebrovascular disease and another patient group with focal cerebrovascular lesions About a third of the patients with diffuse cerebrovascular disease had entirely normal clinical neurological exams and most of the rest had minimal or isolated signs or deficits of equivocal significance" (p 160) Both patient groups did significantly worse than the control group on all of the neuropsychological tests Although some expected differences in pattern of neuropsychological deficit were associated with right versus left hemisphere focal lesions the diffuse cerebrovascular group performed similarly to the combined (left and right hemisphere) focal group on all tests Furthermore the patients with diffuse cerebrovascular disease who had normal clinical exams were just as impaired on all the neuropsychological tests as were the patients with abnormal clinical findings This latter result in particular suggests that these tests are sensitive to the subtle behavioral deficits resulting from diffuse cerebrovascular disease In another study Reitan (18) assessed the accuracy of clinical type diagnostic inferences based upon the same test battery He was able to classify correctly 88% of a diffuse cerebrovascular disease group 75% of a focal cerebrovascular disease group 88% of a diffuse traumatic head injury group 81% of a focal trauma group 81% of an intrinsic cerebral tumor group 50% of an extrinsic tumor group and 94% of a multiple sclerosis group Filskov and Goldstein (8) obtained equally high classification rates in a similar study of patients having cerebrovascular disease and other neurologic conditions

Despite the demonstrated sensitivity of the Halstead Reitan battery to the behavioral deficits caused by cerebrovascular disease the results of such testing in the present study must be considered equivocal The PXE patients showed some generalized impairment relative to the normal controls and at least two of these patients were quite generally impaired in their neuropsychological functioning Nevertheless except for these two individuals (7% of the total group) our PXE patients did not have deficits which approach the magnitude of those found among patients known to have cerebrovascular disease

Considering that neurasthenia is a mental symptom most frequently associated with PXE in the literature one might expect that PXE patients would have trouble completing our seven hours of

Table III *Kendall's ordinal correlations between PXE physical measures and neuropsychological summary measures*

	τ
Average impairment rating	
Skin involvement	10
Eye (streaks) involvement	11
Peripheral arterial involvement	– 10
Halstead impairment index	
Skin involvement	14
Eye (streaks) involvement	– 05
Peripheral arterial involvement	– 19

Available for only 22 of the 27 patients

continuous testing and would do especially badly on tests requiring speed strength and sustained concentration and effort Although we know of no specific test or operational definition of neurasthenia many of the tests used in this study involve a time pressure and do require sustained effort Because the tactual performance test and trail making test are particularly demanding in these respects the PXE group s poor showing on these tests might be consistent with the hypothesized neurasthenia However this evidence must be weighed against the normal performances of the PXE group on many other timed tests (e g the performance subtests of the WAIS) Furthermore the PXE patients tolerated the day long neuropsychological evaluation equally as well as did the controls Contrary to previous reports on other PXE patient groups our patients did not show conspicuous mental fatigue in connection with physical and mental effort (3)

Previous investigators also have suggested that many patients with PXE have impaired memory Carlborg et al (3) reported a 31% incidence of etfulness in their patients Apparently this fig is based upon patients subjective impressions ich can be misleading (13) We were able to demonstrate objective memory deficits in four patients with PXE and in one control subject and the group differences on the story memory test are not statistically significant

The personality test results were within normal limits for 18 of the 27 patients with PXE Only two had MMPI profiles reflecting psychiatric disturbances greater than those shown by any of the controls These are not the two patients who were markedly impaired on the neuropsychological test battery Furthermore in these individuals with significant psychiatric disturbances current life stresses and chaotic childhood backgrounds probably have had greater etiologic significance than the PXE The only statistically significant MMPI difference between the PXE and control groups occurred on the hypochondriasis scale Slight elevations on this scale could well reflect realistic concerns of the PXE patients about current physical status and prognosis concerns which are understandably greater than those of control subjects who have no physical illness

According to Carlborg et al (3) there is some relationship between severity of mental disturbance and severity of other PXE symptoms Those of their patients who could still work normally had relatively mild forms of the disease In our group the relationships between neuropsychological summary measures and indices of skin eye and peripheral arterial involvement did not reach statistical significance but neither did most of the latter indices correlate significantly with each other Although the *presence* of characteristic skin lesions and angioid streaks is an almost invariable finding in adult patients with PXE there may be no uniform relationships between the *degrees* of these and the other symptoms of the disease For example some patients may have especially severe skin lesions relative to their eye involvement while other patients may show the opposite pattern The ages of our patients may also contribute to the modest relationships among the degrees of their various PXE symptoms Previous authors (1, 6) have suggested that PXE skin lesions typically commence around the age of 15 whereas other symptoms are found less consistently and tend to appear during young adulthood (e g diminished peripheral pulses and impaired vision) or after middle age (serious cardiocerebrovascular involvement) The fact that the average age of patients in previous investigations is more than 15 years above the mean age of our group (3 6) may account for the apparent discrepancy between their observations and our test findings

In 20 of our PXE patients abnormalities in carotid flow patterns were sought using Doppler waveform measurements over the common carotids The findings were all normal except for a borderline study in one individual These results support those of the neuropsychological test battery in suggesting that there is no marked involvement of PXE cerebral vessels in this age group Since the appearance of other PXE symptoms is to some extent age related we cannot rule out the possibility that more abnormalities would be found in an older group of PXE patients The very mild deficits found in our younger patients also suggest that further neuropsychological research with older PXE patients is warranted In such studies comparisons with age matched control groups are essential if any apparent abnormalities are to be attributed to PXE

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Fig 1 Zonular cataract in the peripheral cortex of the left lens

phosphatases liver function tests and urine analyses were normal as was the electrophoretic serum protein pattern TSH TRH calcitonin thyroxine serum gastrin and serum aldosterone were normal The level of serum para thyroid hormone was at the lower limit of the normal ge 1 1-1 3 ng/ml (1 1-2 5) (1) ECG was not remarka apart from a slightly prolonged QT interval (0 50 sec) rays of the skull and long bones were normal X rays of e hands showed signs of osteoarthritis

Ophthalmological findings

Visual acuity with best refraction was 0 7 (20/25) for the right eye and 0 4 (20/50) for the left Slit lamp microscopy revealed a zonular cataract in the peripheral cortex of both lenses The cataract was somewhat more dense in the posterior than in the anterior cortex and was more pronounced in the left eye The zonular opacity had a radial appearance and was more dense at the anterior and posterior poles within a circular area with a diameter of about 2 mm (Fig 1) This thin zonular opacity was located *centrally approximately 0 15 mm from the lens surface* The lens cortex outside the zonular opacity was transparent and appeared normal Inside the zonular cataract of both lenses three zones of discontinuity and a peripheral cuneiform opacity were seen in the deep cortex The lens nuclei were clear but yellowish Apart from the lens findings no pathological changes were detected in the eyes

Treatment and subsequent course

The patient was treated with dihydrotachysterol (Dygratyl* 0 4 mg/day) and calcium gluconate (Calcium Sandoz* 2 g/day) After the institution of therapy the serum concentrations of calcium phosphate and magnesium normalized as did the urinary excretion of calcium and phosphate She has been free from tetany

Prevalence of hypocalcaemia in a health screening programme

A medical screening is offered regularly to employees of the Stockholm City and County Council From July 1971 to July 1973 15903 employees (20–63 years) were examined at such health check ups The medical controls are performed at various personnel surgeries and comprise a physical examination and laboratory investigation including conventional chemical analyses of blood and urine samples using Auto-Chemist. All data are organized for computer analysis The distribution by age and sex is given in a previous publication (6) Nine of the subjects had a single serum calcium registration of less than 2 10 mmol/l giving a prevalence of 0 6 ‰ None of the subjects proved to have primary hypoparathyroidism The hypocalcaemia was related in all cases to other disorders (Table I)

DISCUSSION

The clinical diagnosis of idiopathic hypoparathyroidism is based on the classical criteria outlined by Drake et al (8) a low concentration of calcium and a high concentration of inorganic phosphate in serum chronic tetany and absence of history or evidence of surgical or other trauma in the parathyroid region There must be no roentgenographic evidence of osteopenia (rickets or osteomalacia) and other causes of hypocalcaemia (such as renal insufficiency malabsorption and conditions with alkalosis) must be ruled out The case reported here fulfilled all these criteria and the diagnosis of sporadic idiopathic hypoparathyroidism was confirmed by low serum levels of parathyroid hormone There was no evidence of a familial form of disease or of other endocrine disturbances and there were no signs of concomitant autoimmunological traits (e g Addison s disease pernicious anaemia) which have been reported in some cases (3 17 18 20)

Tetany latent or manifest has been said to be the most characteristic feature of the disease occurring in almost 80% of the cases reviewed by Steinberg and Waldron (21) Convulsions occurred in approximately 50% being more common in young people (14) In the present case tetany and convulsions had occurred 12 years before hypocalcaemia was identified These symptoms seem to have been absent for several years in the interval becoming evident again shortly before the diagnosis was established The manifestations of neuromuscular irritability in parathyroid deficiency appear to be less frequent and dramatic in elderly persons and this has been suggested as one explanation of the low prevalence of idiopathic hypoparathyroidism among the middle aged and elderly (14)

The relationship between cataract and hypoparathyroidism was first demonstrated experi

Table I *Clinical data on 9 female subjects with hypocalcaemia (<2 10 mmol/l) detected in a health screening*

Pat no	Age (y)	Serum concentrations Calcium (mmol/l)	Phosphate (mmol/l)	Albumin (g/l)	Creatinine (μmol/l)	Previous history
1	61	2 03	1 8	42	159	Chronic pyelonephritis
2	60	2 08	1 9	40	186	Chronic pyelonephritis
3	59	2 00	2 0	43	248	Chronic pyelonephritis
4	59	1 98	2 1	42	256	Chronic pyelonephritis
5	*57*	*2 01*	*0 8*	*43*	*80*	*Osteomalacia*
6	56	1 99	0 7	42	80	Osteomalacia
7	57	2 06	0 8	43	71	Thyroidectomy
8	60	2 03	0 8	42	80	Thyroidectomy
9	55	2 00	0 9	44	71	Thyroidectomy
Normal range		2 20–2 60	0 7–1 6	37–52	50–110	

mentally by Erdheim in 1906 (9) In the idiopathic type lenticular opacities are found in 58% of the cases (19) These opacities may well be even more frequent as in many of the reported cases the ocular examination was not complete The lenticular changes are bilateral and cortical Generally the opacities are of the subcapsular or lamellar type indicating a malfunction of the lens epithelium during a certain period of life The more peripheral the zone of opacities the more recent was the period of lens damage The distance of the opacity from the lens surface can be used to date the onset of cataract formation (5) Growth of the lens radius amounts to approximately 0 014 mm per year In the present case the onset of cataract can be calculated to about 11 years ago which conforms well with the onset of neuromuscular symptoms 12 years ago The most peripheral cortex was clear which indicates that the lens epithelium had functioned *normally during the last 10 years As it is well* documented that hypocalcaemia causes malfunction of the lens epithelium and lens fibres (10) the calcium level in the aqueous humor blood could not have been extremely abnormal during that period

The insidious onset of symptoms in idiopathic hypoparathyroidism probably accounts for the frequent delay in the correct diagnosis (7) Retrospectively the present case represents a clear classical establishment of diagnosis The relative absence of symptoms together with the apparent cessation of cataractal development during the latter 10 years point to the presence of less severe hypocalcaemia during this period It is tempting to suggest that this might have to do with the onset of menopause a condition which several authors associate with an increase in serum calcium levels (13 15 16 22)

The clinical impression that hypocalcaemia is a rare condition was confirmed in the present analysis of 15903 persons attending a health screening programme Severe hypocalcaemia (<2 10 mmol/l) was found in only 0 6‰ and in no case could the hypocalcaemia be attributed to idiopathic hypoparathyroidism Thus the rarity of the condition and the less pronounced neuromuscular symptoms together with the occurrence of age related degenerative changes in the lens make it especially important to recollect the diagnosis in elderly patients Some reports suggest that progression of the cataract can be arrested by treatment (11) and the possible occurrence of hypocalcaemia should be considered in cases with bilateral subcapsular cataract

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Acute Interstitial Nephritis during Treatment with Penicillin and Cephalothin

Nils Milman

From Department of Infectious Diseases M Rigshospitalet Copenhagen Denmark

ABSTRACT A case of non-oliguric acute interstitial nephritis *during treatment with ampicillin*, benzylpenicillin and cephalothin is reported There were symptoms of drug hypersensitivity, including fever exanthema, eosinophilia and elevated serum IgE Renal biopsy showed marked interstitial edema and infiltration with numerous eosinophils, some mononuclear cells and giant cells, and scattered tubular damage, but normal glomeruli and vessels There was no pathological deposition of immunoglobulins or complement in the renal tissue Renal function recovered after withdrawal of antibiotics and treatment *with steroids The findings suggest a drug* induced, hypersensitivity mediated pathogenesis of the renal lesions, with participation of both humoral and cell mediated immunological reactions

The β lactam antibiotics ampicillin benzylpenicillin and cephalothin are relatively non toxic and even in high doses have no or only very slight renal toxicity in man (15 17) Hypersensitivity reactions can occur *with all penicillin related antibiotics (11 17) and may* although uncommonly involve the kidney as manifest by acute diffuse interstitial nephritis (AIN)

CASE REPORT

A 15-year-old boy was admitted to hospital after 10 days of unexplained fever The patient had on previous occasions received penicillin orally without allergic reactions although there was a family history of penicillin allergy *The main features of the history are depicted in Fig 1*

On admission the patient was febrile but appeared unaffected by the illness There was distinct neutrophilia and a blood culture yielded growth of Streptococcus Milleri On hospital day 2 treatment was started with gentamicin 180 mg/24 h and cephalothin 4 g/24 h Cephalothin was replaced by ampicillin 2 g/24 h on day 5 The investigations did not reveal the focus of the infection the fever subsided and all antibiotic treatment was stopped on day 10 Serum creatinine was normal before and after gentamicin administration there was no proteinuria a urine culture was negative and the urine sediment was normal On day 11 the temperature again rose and treatment with benzylpenicillin 12 g/24 h (20 000 000 IU/24 h) and streptomycin 1 g/24 h was started However the patient was persistently febrile and on day 25 a diffuse erythematous morbilliform itching exanthema appeared which indicated treatment with prednisone on suspicion of penicillin allergy whereafter the fever immediately subsided On day 30 benzylpenicillin was discontinued and replaced with cephalothin 4 g/24 h and tetracycline 2 g/24 h which were both withdrawn on day 33 Prednisone was discontinued on day 33 whereupon the fever recurred and the exanthema increased Hereafter serum creatinine rose to a maximum of 0 20 mmol/l and creatinine clearance decreased to 26 ml/min Urine sediment contained 30–40 leucocytes per high power field numerous hyaline casts granular casts leucocyte casts and mucous threads but no erythrocytes or erythrocyte casts There was minimal proteinuria, ranging from 0 1 to 0 5 g/24 h and protein electrophoresis on concentrated urine showed small amounts of albumin urine cultures were negative

An i v urography demonstrated bilaterally enlarged kidneys with normal calyces and no obstruction There was distinct eosinophilia and prednisone treatment was resumed hereafter the fever and eosinophilia subsided However on the suspicion of recurrent septicemia cephalothin 3 g/24 h was given from day 38 to 48 Resumption of cephalothin therapy was immediately followed *by a rise in temperature and increasing eosinophilia*. A percutaneous renal biopsy was performed on day 41 culture from the biopsy was negative

After the prednisone dose had been increased the patient became afebrile the eosinophilia and exanthema disappeared and renal function improved rapidly On day 48 the serum creatinine was 0 08 mmol/l and creatinine clearance 90 ml/min. The prednisone dose was gradually reduced and treatment stopped on day 102 At later controls the patient appeared well with complete recovery of renal function normal urine sediment and no proteinuria.

No hypotensive episodes were observed and there were no signs of hyperhemolysis or septic emboli Repeated

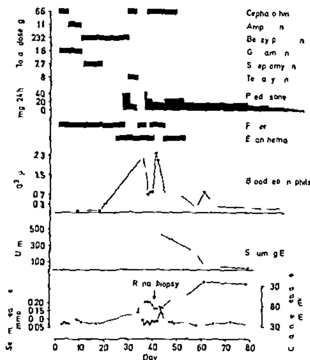


Fig 1 Relevant clinical data on the patient



Fig 2 Heavy interstitial infiltration with granulocytes and mononuclear cells. Tubular epithelium with degenerative changes and patchy necrosis (×108)

determinations of serum antistreptolysin O and antihyaluronidase showed normal constant titers.

Indirect immunofluorescence studies with serum showed no organ unspecific or granulocyte specific antinuclear antibodies of IgG class and the anti DNA antibody titer was normal. Immunofluorescence studies of punch biopsy from skin exanthema demonstrated granular deposition of Cl_q and C4 in the dermal-epidermal junction and the vessel walls but no IgG, IgA, IgM, IgD, IgE or fibrinogen. LE cell tests, Wassermann, Waaler Rose and rheumatoid factor tests were negative and there was no cryoglobulinemia. Direct Coombs test was transiently slightly positive. Plasma C3 and C4 were normal. Plasma IgA and IgM were in the normal range while IgG was transiently elevated. Serum IgE measured by radioimmunosorbent test (Phadebas®) was elevated on day 41, 478 U/ml (normal 31–370), and then gradually decreased to normal values (Fig 1). Serum contained penicilloyl specific antibodies of IgE class (measured by a radioallergosorbent test).

The kidney biopsy specimen was fixed in Lilly's fluid. Paraffin embedded tissue was cut into 2–3 µm thick sections and stained with hematoxylin-eosin, silver methenamine hematoxylin and Lendrum's fibrin stain. The biopsy consisted of cortical tissue containing approximately 70 glomeruli which all appeared normal. The most impressive lesion was found in the interstitial tissue showing edema and heavy cellular infiltration with large numbers of eosinophils, neutrophils and histiocytes and a few lymphocytes and plasma cells (Fig 2). In the most severely affected areas there was marked degeneration or necrosis of the tubular epithelium with destruction of the tubular basement membranes. A few granulocyte casts were seen, erythrocyte casts were absent. Scattered in the interstitium an amorphous eosinophilic material was observed which reacted positively with Lendrum's stain for fibrin, surrounded by multinucleate giant cells with the nuclei arranged around the periphery and at one pole. These lesions had the appearance of small granulomas (Fig 3). One of the interlobular arteries showed discrete changes with deposition of Lendrum positive material in the wall but without cellular infiltration, the other blood vessels appeared normal (Dr C Brun).

Direct immunofluorescence studies were carried out on cryostat sections from part of the biopsy. They disclosed equivocal focal staining for IgG in the glomerular mesangium and along the glomerular basement membranes and very slight staining for C3 subendothelially and intramurally in the arteries and in the interstitium. Stainings for IgA, IgM, IgD, IgE, properdin, Cl_q, C4 and fibrinogen were negative (Dr S Larsen).

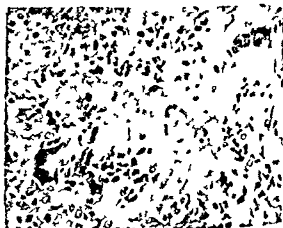


Fig 3 Multinucleate giant cells in the renal interstitium (×208)

DISCUSSION

The sequence of events in the present case with fever exanthema eosinophilia positive Coombs test elevated serum IgE penicilloyl specific IgE antibodies in serum and beneficial response to steroid treatment strongly suggests a drug induced hypersensitivity reaction (11) The patient was treated with β lactam antibiotics for 23 days before the occurrence of exanthema and probably developed hypersensitivity against both benzylpenicillin and cephalothin Hypersensitivity against these two antibiotics is a well recognized feature (8 11 15) and cross allergy between them although uncommon has also been reported (15)

AIN is a clinico-pathological entity which in many cases has been attributed to treatment with a variety of drugs especially penicillin related antibiotics such as methicillin (3 4 10) benzylpenicillin (3) ampicillin (14) and cephalothin (5) The causative factors are unknown but there are several features suggesting an immunologic pathogenesis Many of the reported cases displayed symptoms of drug hypersensitivity including fever exanthema and eosinophilia (3 4 5 14) In methicillin induced AIN methicillin haptenic groups and IgG have been demonstrated in the renal interstitium and in the tubular and glomerular basement membranes (3) This condition has also been associated with circulating antitubular basement membrane antibodies (4) and deposition of methicillin antigen (4) IgG and C3 along the tubular basement membranes (4 7) The renal pathology usually demonstrates marked interstitial cellular infiltration with lymphocytes histiocytes plasma cells eosinophils and neutrophils together with scattered tubular necroses while glomeruli and blood vessels appear normal (3 4 5 7 10 14)

The renal pathological findings in the present case are consistent with a drug induced AIN caused by the β lactam antibiotics ampicillin benzylpenicillin and cephalothin Although the patient received a course of aminoglycoside therapy it seems unlikely that this could be responsible for the renal lesions Aminoglycoside nephrotoxicity is characterized by tubular damage with only slight interstitial changes (9) the doses were relatively low and serum creatinine was normal during administration and after withdrawal of gentamicin Cephalothin in large doses in animals produces only slight changes in the proximal tubular epithelium but no interstitial changes (13) Consequently the renal lesions could not be ascribed to a toxic effect of cephalothin per se

The pathogenesis of the renal lesion in the present case is unclarified but the clinical picture and the renal interstitial infiltration with lymphocytes plasma cells and eosinophils are suggestive of a hypersensitivity reaction

In animal experiments it is possible to produce antibody mediated tubular and interstitial renal immune complex diseases and there is compelling evidence that cell mediated hypersensitivity can account for some forms of interstitial nephritis (2)

According to Coombs and Gell (6) allergic reactions responsible for hypersensitivity and disease can be classified into four clinically recognized types and it is likely that several types of immunological reactions participate to various degrees in the same patient (6)

The presence of numerous eosinophils in the blood and renal interstitium concomitantly with a distinctly elevated serum IgE are strong indicators for an immediate type I immunological reaction (12) although IgE carrying plasma cells were not observed in the renal biopsy

Furthermore the interstitial infiltration with lymphocytes histiocytes and plasma cells suggests a cell mediated type IV immunological reaction (6) It is possible that the cell mediated hypersensitivity is directed against a drug derived hapten and a structural protein in the renal interstitium or the tubular basement membrane Thus Baldwin et al (3) demonstrated the presence of penicilloyl haptenic groups along the tubular basement membranes and in the interstitium

The positive Coombs test is compatible with a cytotoxic type II immunological reaction (6) involving the red blood cells This phenomenon is often encountered during benzylpenicillin and cephalothin treatment (1 15) and usually is of minor importance Accordingly there were no symptoms of increased hemolysis in the present case On the other hand there was no evidence of a type II immunological reaction in the kidneys as judged by the absence of pathological depositions of immunoglobulins and complement However other authors have suggested that a type II reaction plays a role in methicillin induced AIN Baldwin et al (3) found IgG (but no C3) distributed in a pattern similar to that observed for the penicilloyl haptenic groups in the renal parenchyma and considered in view of

the absence of glomerular damage that the renal lesions were not mediated by circulating antigen–antibody complexes Border et al (4) demonstrated the deposition in a linear pattern of IgG, C3 and a methicillin antigen along the tubular basement membranes together with the presence of circulating antitubular basement membrane antibodies The formation of these antibodies is perhaps a secondary feature stimulated by damage and release of tubular basement membrane components

Immunofluorescence studies did not demonstrate any distinctly abnormal deposits of immunoglobulins or complement in the renal tissue These findings and the normal serum levels of C3 and C4 the minimal proteinuria the absence of erythrocyturia, and the normal glomerular morphology argue against an immune complex mediated type III immunological reaction This is consistent with the observations reported by other authors (3 4 5 7 10 14)

The presence of multinucleate giant cells has not previously been reported in connection with benzylpenicillin induced AIN but granuloma like i of histiocytes in renal biopsies from subjects ith methicillin associated AIN have recently been described (10) Giant cells are formed by fusion of histiocytes and are found in high turnover granulomas (16) The formation of granulomas can be induced by immunological mechanisms involving both antigen–antibody reactions and cell mediated immunological reactions (16 18)

Although the findings in the present case are highly suggestive of a drug induced hypersensitivity mediated renal lesion it should be admitted that conclusive evidence is lacking as the hapten in the renal tissue was not identified

Renal function recovered completely after the withdrawal of benzylpenicillin and cephalothin The prognosis in drug induced AIN is generally favourable (3 5 7 14) and recovery is more the rule than the exception

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Persistent Hypothalamic-Pituitary Insufficiency Following Acute Meningoencephalitis

A Report of Two Cases

Erik Hagg Lennart Åstrom and Lars Steen

From the Department of Internal Medicine University Hospital Umeå and the Department of Internal Medicine County Hospital Sollefteå Sweden

ABSTRACT This report concerns two patients, a 43-year-old woman and a 53-year-old man, who developed clinical as well as laboratory signs of permanent gonodal and thyroid failure following an acute intracranial infection—in the woman a meningoencephalitis of unknown origin, and in the man an encephalitis caused by Coxsackie B 5 Endocrine investigations were compatible with hypothalamic pituitary dysfunction with some of the results favoring a hypothalamic lesion Perhaps hormone deficiency of hypothalamic and/or pituitary origin is a more common sequel of acute meningoencephalitis than has hitherto been reported

Hypothalamic and pituitary insufficiency has diverse causes In meningoencephalitis caused by microorganisms the hypothalamus and pituitary may be affected but deficient hormonal secretion from these regions has not been reported very often and almost exclusively following tuberculous meningitis (cf 21 23) Hypothalamic pituitary dysfunction mainly diabetes insipidus has been described in three neonates with meningitis caused by group B β-hemolytic streptococci (14 18) Abramsky et al (1) reported a 68-year-old man with transient diabetes insipidus as a complication of pneumococcal meningitis In a review of 42 cases of diabetes insipidus Jones (10) mentioned one patient with chronic meningitis and encephalitis of unknown origin and three patients with syphilis of whom two had evidence of disease of the central nervous system Hypopituitarism has also been described in some patients with abscess formation in the pituitary region sometimes associated with a pituitary tumor or as a complication of sphenoid sinusitis (cf 7 15) In addition diabetes insipidus has been reported in a few cases of sinusitis without pituitary abscess formation (3 27)

Since hypothalamic or pituitary hormonal deficiency apart from diabetes insipidus following acute non tuberculous meningoencephalitis in adults does not appear to have been noticed in the literature we considered it of interest to report two such cases

ENDOCRINE STUDIES

Serum cortisol concentration was estimated by radioimmunoassay (Gammacoat Clinical Assays Inc) Total thyroxine in serum was assayed by competetive protein binding technique (20) and thyrotrophin (TSH) in serum according to Odell et al (17) Serum luteinizing hormone (LH) follicle stimulating hormone (FSH) and growth hormone (GH) were determined by radioimmunosorbent technique (24 25) Serum testosterone level was assayed by radioimmunoassay (2) Porter Silber chromogens and 17 ketosteroids in urine were measured by the methods of Silber and Porter (22) and Hamburger (9) respectively

The insulin tolerance test was performed in the morning in fasting state and 0 10–0 15 U/kg of insulin was given i v In both patients the blood glucose concentration fell below 2 0 mmol/l associated with profuse sweating Blood for cortisol and GH assays was drawn at zero time and 15 30 60 90 and 120 min after insulin administration In vasopressin test 10 IU of lysine vasopressin (Postacon®) was given i m Serum cortisol concentration was determined before and 30 and 60 min after injection Thyrotrophin-releasing hormone (TRH) and LH releasing hormone (LH RH) tests were performed as follows 200 µg TRF (Roche) and 100 µg LH RH (Hoechst) were given i v Blood samples for measurements of TSH and of LH and FSH respectively were taken before and 20 and 60 min afterwards

Table I *Serum hormone values in the patients*

	Normal values	Pat 1	Pat 2
Thyroxine (nmol/l)	79–158	52 65 54	36
Free thyroxine index	72–160	41 53 43	30
TSH (mU/l)	1 0–8 0	2 8–5 7	3 3 5 2
Testosterone (µg/l)	♀ 0 40–0 80 ♂ 4 0–10 0	<0 11	 2 1
LH (µg/l)	0 4–3 0	0 18	0 13
FSH (µg/l)	0 5–3 0	0 70	0 35

REPORT OF CASES

Case 1

The patient a 43 year-old housewife with four children (the youngest born in 1970) has no family history of endocrine diseases She had been essentially healthy until Dec 26 1972 when she fell acutely ill with headache nausea and vomiting On the following day high fever (max 39 4 C) and deterioration of vision occurred and she was admitted to the Department of Internal Medicine her local hospital

e patient was somewhat drowsy on admission but id Examination revealed normal eye grounds central scotomas on both eyes with greatly impaired visual acuity *which was interpreted as retrobulbar neuritis Except for* these visual signs the neurological examination was normal Cervical rigidity absent on admission was noted two days afterwards Repeated (three) lumbar punctures disclosed an increased pressure (285 mm of water) and a pleocytosis (almost 400 cells/mm³) consisting mainly of polymorphonuclear cells Protein content of cerebrospinal fluid was increased to 277 mg/100 ml and glucose concentration was slightly reduced (about 40 mg/100 ml) There was no xantochromia. Bacteria could not be detected on direct examination and cultures of spinal fluid for bacteria (including tubercle bacillus) and virus did not show any growth EEG disclosed a slight general abnormality An *X ray of the skull was normal Maximum ESR* was 81 mm/hour

The patient was treated with ampicillin streptomycin isoniazid and ACTH and a rather rapid amelioration occurred The fever disappeared on the sixth day after admission On the third hospital day urinary output was 5 5 l/24 hours Urinary concentration power was not tested Administration of vasopressin decreased the polyuria. When the patient left hospital after one month she was feeling well her visual impairment had greatly improved and EEG was normal Treatment with streptomycin and isoniazid was continued for an additional month but no further medication was given

After the disease in Dec 1972 the patient has had amenorrhea (last menstruation in Dec 1972) libido has diminished and she has suffered from tiredness In March 1973 paresthesias weakness and edema of the hands started and a hypertension was detected with a peak value of 210/130 mmHg The hypertension has been treated with propranolol (Inderal®) 80 mg twice daily since April 1974 In 1974 she gained approximately 10 kg and began to notice puffiness of her face and eyelids When examined at the Medical Department of her local hospital during May *June 1973* the serum level of protein bound iodine was low (2 7–2 8 µg/100 ml) Serum LH concentration was 0 60 µg/l (near the lower border for women of fertile age) and serum FSH 0 90 µg/l (normal value) Urinary estrogen levels were very low (estrone+estradiol 1–2 µg/24 hours) Temporary oral treatment with conjugated estrogens (Promant®) during 1975 did not induce menstrual bleeding In the same year treatment with levothyroxine sodium (Levaxin® 0 1 mg/day) was given for 3 months yet her symptoms did not disappear

During 1976 the patient was further treated at the Department of Internal Medicine of the local hospital and of Umeå University Hospital Physical examination revealed *a moderate obesity and thinning of axillary hair but the* skin was without abnormalities The thyroid gland was not palpable BP was normal without propranolol medication Gynecological examination was uneventful and a routine neurological investigation was normal Examination of the ocular fundi showed a slight pallor of the optic discs and there was a homonymous left lower quadrantic anopia. X ray examination of the pituitary fossa and EMI scanning of the brain were normal

ESR varied between 21 and 38 mm Hb concentration was normal Serum levels of sodium potassium and creatinine were normal An oral glucose tolerance test was within normal limits Basal metabolic rate was −18% *Cholesterol value in serum was 7 0 mmol/l* Antithyroglobulin antibodies could not be detected in the serum Urinary osmolality was normal under water deprivation (960 mosm/kg)

As shown in Table I the patient had low values of both serum thyroxine and free thyroxine index Serum TSH level was normal and after TRH administration (Table II) TSH showed a normal rise but with a tendency to a delayed response During the insulin tolerance test the peak serum cortisol value was on the low side in contrast to the quite normal elevation during the vasopressin test. Hypoglycemia did not induce any significant increase in serum GH concentration Urinary excretion of Porter Silber chromogens was normal (about 15 µmol/24 hours) but the urinary content of 17 ketosteroids showed low borderline levels (about 10 µmol/24 hours) Serum testosterone and LH concentration were significantly reduced There was a significant increase in both LH (of 159%) and FSH serum levels (of 67%) 60 min after LH RH administration

Substitution therapy has just started (with cortisone acetate levothyroxine sodium estradiol and norethisterone) and therefore the effects of this treatment cannot yet be evaluated

Case 2

A 53-year-old foreman with no heredity for endocrine disorders Apart from nasal polyps he had been well *In* the beginning of Oct 1973 the patient complained of a severe headache nausea vomiting and high fever (39.3 C)

Table II *Effect of hypoglycemia vasopressin and TRH on serum cortisol GH and TSH levels in the patients*

	Normal values	Pat 1	Pat 2
Insulin tolerance test			
Cortisol (nmol/l)			
Basal level	>280	228	322
Peak level	>600	539	751
GH (μg/l)			
Basal level	<5	0 64	<0 52
Peak level	>10	1 14	0 68
Vasopressin test			
Cortisol (nmol/l)			
Basal level	>280	457	
Peak level	>500	844	
TRH test			
TSH (mU/l)			
0 min	TSH	4 0	5 2
20 min	increase	10 7	14 3
60 min	2–25	12 7	14 6

and was treated at home by the general practitioner After 6 days doxycycline (Vibramycin®) was given orally and the patient improved

In the beginning of Nov an intensive headache reappeared and the patient was admitted to the Department of Infectious Diseases at Umeå University Hospital On admission his mental state was unaffected Routine neurological examination disclosed no gross abnormality and there was no cervical stiffness The ocular fundi were normal. Lumbar puncture revealed normal pressure (130 mm of water) a clear colourless cerebrospinal fluid with 14 mononuclear cells/mm³ and a slightly increased protein concentration (59 mg/100 ml) Spinal fluid cultures were positive for Coxsackie B 5

A few days after admission his temperature rose from about 37°C to 38 5°C and the patient complained of double vision A sixth nerve paresis of both eyes was found EEG revealed a slight unspecific abnormality over the posterior parts of both hemispheres Both echoencephalography and skull X ray were normal except for a thickening of the lining membranes of the right maxillary sinus and of the ethmoid cells ESR was 56–24 mm/hour

Treatment began with oral azidocillin (Globacillin®) and nose drops and the patient improved He left hospital after nearly four weeks and after an additional three months the diplopia had disappeared completely

Since the disease in 1973 the patient has suffered from tiredness In 1975 he also noticed intolerance to cold dryness of the skin decreased sweating and disappearance of hair on the trunk and extremities Furthermore libido decreased and ejaculation was abolished In April 1974 serum thyroxine level was somewhat low 66 8 nmol/l as was the free thyroxine index 41 Serum TSH concentration was normal 5 2 mU/l In 1975 he had an episode of bacteriuria and was treated with antibiotics

In 1976 the patient was referred to the Department of Internal Medicine at Umeå University Hospital by the general practitioner because of low blood Hb level (about 100–110 g/l) On admission his general condition was good Body hair was reduced in the axillae and the pubic region The skin was pale somewhat dry and atrophic There was no gynecomastia The thyroid gland was not palpable The pulse was slow (50/min) and the BP 145/90 mmHg The testes and the prostate were of normal size A thorough neurological examination did not disclose any thing abnormal Ocular fundi visus and visual fields were normal X-ray of the skull showed a normal pituitary sella The patient did not consent to an EMI scanning of the brain I v pyelography was normal except for one small stone (1×2 mm) on both sides located in the renal papillae

ESR was 32–23 mm/hour and Hb level 110 g/l Serum iron concentration was within normal limits Cholesterol value was 10 8 mmol/l Test for antithyroglobulin antibodies was negative Serum sodium potassium calcium and phosphate concentrations were normal Serum creatinine value was somewhat increased 113 μmol/l Urinary concentration ability was reduced to 650 mosm/kg The urine contained increased amounts of leucocytes though bacterial urine cultures were negative

Serum level of thyroxine and free thyroxine index were low (Table I) Basal serum TSH concentration was normal as was the rise of TSH after TRH administration (Table II) except for a tendency to delayed response During the insulin hypoglycemia test the increase in serum cortisol level was normal but there was no significant rise of serum GH concentration Serum testosterone LH and FSH levels were all low However the increase in LH (of 262%) and in FSH values (of 51%) 60 min after LH RH administration appears to be essentially normal (26) Urinary excretion of Porter Silber chromogens was normal 10–25 μmol/day but the excretion of 17 ketosteroids showed low borderline levels 11–15 μmol/day

The patient was treated with levothyroxine sodium (Levaxin®) and injections of testosterone (Testoviron-Depot®) with a marked improvement of his symptoms

DISCUSSION

The woman presented here thus showed clinical and laboratory signs of gonadal and thyroid insufficiency There were also laboratory findings of deficient secretion of GH and of a slightly impaired hypothalamic pituitary adrenal system In addition the patient had clinical signs of a transient diabetes insipidus during the acute illness It is beyond doubt that these hormonal abnormalities were caused by the preceding acute meningoencephalitis which in accordance with her visual impairment engaged the basal portions of the brain The causative agent of the infection is not known but might probably be of viral origin

The male patient had an encephalitis caused by Coxsackie B 5 Following this infection he de

veloped signs of hypogonadism and of thyroid failure He also had impaired GH secretion during insulin tolerance test but an intact hypothalamic-pituitary adrenal axis

As to the localization of the lesion causing deficient hormonal secretion in these two patients the results of the TRH test favor a hypothalamic rather than a pituitary lesion The typical response during a TRH test in patients with hypothalamic lesions is a rise of serum TSH but of a delayed type i e the 60-minute level is higher than the 20-minute level (8) Patients with pituitary hypothyroidism typically show an impaired or absent response (8 11) However the TRH test does not seem to be entirely reliable in distinguishing between hypothalamic and pituitary hypothyroidism since some patients with pituitary tumors and hypothyroidism have shown a dalayed or even a normal response to TRH (5, 12 19) A normal rise of serum cortisol values during hypoglycemia (as in insulin tolerance test) implies an intact hypothalamic pituitary adrenal system (4) Consequently patients with hypothalamic lesions may show an insufficient increase in cortisol during insulin hypoglycemia test On the other hand h patients can exhibit a normal rise of serum ortisol levels during a vasopressin test (4) This was the situation of our female patient which might indicate a predominantly hypothalamic lesion The LH RH test does not appear to be able to differentiate between hypothalamic and pituitary causes of hypoganadism (6 13 16)

Concerning the time relationship between the intracranial infection and the commencement of endocrine dysfunction our both patients had laboratory signs of thyroid failure within six months after infection In addition the woman has had persistent amenorrhea following the meningoencephalitis and showed very low values of urinary estrogens five months after the infection

It is not known how often hormonal deficiencies occur during and after an acute meningoencephalitis but it is reasonable to assume that the incidence may vary with the type of causative agent as well as with the extent of the brain lesion Both patients reported here had neurological symptoms from the basal regions of the brain It is possible that only those patients with meningoencephalitis giving focal neurological symptoms will develop hormone dysfunction It may be that hypothalamic pituitary dysfunction is a more common sequel of acute meningoencephalitis than is reflected in the literature and that the clinical picture of some patients with endocrine insufficiency perhaps might be misinterpreted as an ordinary postencephalitic syndrome

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Coronary Heart Disease— a Possible Risk in Megavoltage Therapy?

Sten Rasmussen Martin Døssing and Sven Walbom Jørgensen

From Medical Department C Diakonissestiftelsen the Radiumcenter and Finseninstitute Copenhagen Denmark

ABSTRACT A 21 year old man died of an extensive anteroseptal myocardial infarction 16 months after receiving megavoltage radiotherapy to a mantle field for Hodgkin s disease stage PS IA confined to the midcervical lymph nodes on the left side of the neck Post mortem findings revealed severe atherosclerotic changes in the coronary arteries This case and a review of the literature suggest that irradiation to the heart may induce or accelerate atherosclerosis of the epicardial vessels This should be taken into consideration when starting prophylactic irradiation to the mantle field in patients with Hodgkin's disease stage IA without obvious involvement of the mediastinum Histologic examination of the heart and coronary vessels should be performed in any fatal case after megavoltage therapy involving the heart

Injury to virtually all normal intrathoracic structures including the heart may follow megavoltage radiotherapy to the mediastinum for Hodgkin s disease (7 13) Lesions of the epicardial vessels have occasionally been described We report a case of fatal acute myocardial infarction (AMI) irradiated to a mantle field for Hodgkin s disease stage PS IA

CASE REPORT

A 21 year old man was admitted in Nov 1975 with cardiac arrest which occurred during the last of three severe attacks of chest pain within an hour after vigorous exercise He was resuscitated on admission but died a week later without regaining consciousness ECG showed an extensive anteroseptal infarction and the serum enzymes were increased accordingly

In Jan 1974 he discovered enlarged lymph nodes on the left side of the neck Biopsy showed Hodgkin s disease of the mixed cellularity type Lymphography and laparotomy including splenectomy and biopsies from the liver spleen and paraortic lymph nodes showed no signs of Hodgkin s disease He was finally staged Hodgkin s disease stage PS IA H N S- (Ann Arbor 1971 and Luke s classification 1966)

In July 1974 radiotherapy was given to a mantle field using opposing beams to a total dose of 3 696 rads in 32 days (15) Approximately 60% of the heart was inside the field A month later he was given 3 696 rads to a modified inverted Y field to the promontorium The involved lymph nodes regressed and later controls showed no recurrence of the disease

The patient smoked 20 cigarettes daily he had no previous history of heart disease hypertension or diabetes No analyses of serum lipids were performed but no cutaneous manifestations of hyperlipidemia were seen An older sister has Hodgkin s disease stage IIIB without signs of heart disease or hyperlipidemia (S-cholesterol 5 3 mmol/l S-triglycerides 1 04 mmol/l) The patient s grand father died of AMI at the age of 44 years Otherwise there was no family history of premature ischemic heart disease diabetes hypertension or lipid disorders

Autopsy of the heart showed infarction of the anterior wall and septum A fresh occluding thrombus apparently formed on an atherosclerotic plaque was located in the anterior descending branch of the left coronary artery The right coronary artery and the rest of the vascular system was macroscopically without atherosclerosis There were no signs of Hodgkin s disease anywhere in the body Histological examination of several sections of both the left and right coronary artery showed severe atherosclerosis with intimal proliferation fibrosis calcium deposits cholesterol clefts and fragmentation of the internal elastic membrane (Fig 1) The media and adventitia of the vessels were normal The myocardium of the anterior wall of the left ventricle showed changes compatible with a week old transmural infarction Some interstitial myocardial fibrosis was seen

Reprint requests to M Døssing MD Medical Department C Diakonissestiftelsen Peter Bangsvej 1 DK 2000 Copenhagen F Denmark



Fig 1 Section of the left coronary artery showing severe atherosclerosis and the site on which the thrombus was located

DISCUSSION

Occurrence of coronary heart disease following gavoltage therapy to the mediastinum has been rted in at least eight other patients with Hodg s disease (3 5 6 9 11 12 14) (Table I) The an age of these patients was 28 years (range 15–41) the mean time from irradiation to heart disease was 47 months (range 2–123) and the average dose was 3851 rads (range 1440–5075) All patients were given megavoltage therapy to the thorax either because of obvious involvement of the mediastinum or for prophylactic reasons In all cases a large volume of the heart was irradiated Six patients had AMI leading to death in five Histologic examina-

Table I *Clinical and autopsy findings in patients with coronary artery disease after irradiation for Hodgkin s disease*

Author	Age of the pat (y) at		Sex	Interval (mo) from radiotherapy to		Irradiation dose to mediastinum (rads)	Clinical heart disease
	Death	Disease (after irradiation)		Heart disease	Death		
Dollinger et al (5)	41	31	♂	2	120	1 440+2 470[a]	AMI (inferior)
Prentice (11)	19	18	♂	36?	48?	3 250	Angina pectoris[b]
Cohn et al (3)	15	15	♂	16	16	4 000	AMI (anteroseptal)
Huff & Sanders (6)	21	21	♂	9	9	3 500	AMI (anteroseptal)
Tracy et al (14)	–	35	♀	19	–	5 075	Angina pectoris
McReynolds et al (9)	33	33	♂	100	100	5 000	Sudden coronary death[c]
	–	41	♂	72	–	4 079	AMI (inferior)[d]
Rodgers (12)	–	33	♂	123	–	4 310	AMI (anterolateral)
Present study	21	21	♂	16	16	3 696	AMI (anteroseptal)

[a] Two series of irradiation 6 years apart were administered The patient had AMI after the first treatment
[b] Recent infarction at autopsy 11 months after the start of angina pectoris
[c] Symptoms started with severe substernal pain
[d] The patient developed reinfarction 16 months after the first AMI and survived

tion showed severe atherosclerosis of the coronary arteries and two cases showed thrombosis of the anterior descending branch of the left coronary artery (3 6) Two of the reported cases initially developed angina pectoris Coronary arteriography has been performed in one patient with AMI and in another with angina pectoris extensive coronary artery disease was demonstrated in both (9 14)

A cause–effect relationship between megavoltage therapy and later occurrence of coronary artery disease is difficult to establish Many of the patients subjected to irradiation are also candidates for coronary artery disease for other reasons The interval from irradiation to disease may be so long, that causality between the two events is unlikely The correlation may however have been overlooked because of an overall high incidence of ischemic heart disease Young age no family history of premature coronary heart disease and absence of risk factors together with concomitant findings of well known radiation lesions (such as pericardial fibrosis interstitial myocardial fibrosis and parenchymal pulmonary fibrosis) support the view that irradiation may also be responsible for the observed lesions of the coronary arteries

Animal studies have shown that radiotherapy predisposes for or accelerates the development of atherosclerosis either as an isolated effect or in combination with an atherogenic diet (1 8)

Megavoltage therapy is at present the most effective treatment aiming to cure localized Hodgkin s disease Controversy exists concerning the dose extent and number of fields to be irradiated It is well known that the incidence of complications increases with dose and field size The majority of investigators use extended fields encompassing affected as well as potentially involved areas (7) Others irradiate only involved lymph nodes in Hodgkin s disease stage IA confined to the mid and upper cervical lymph nodes (2) A recent study showed no significant difference in survival between involved and extended field regimens given to patients with Hodgkin s disease stages I and II (4)

These results should be taken into consideration when starting prophylactic irradiation to the mantle field in patients with Hodgkin s disease stage IA without obvious involvement of the mediastinum Chest pain in young patients after radiotherapy should not be ignored Histologic examination of the heart and coronary vessels should be performed in any fatal case after megavoltage radiotherapy

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ANNOUNCEMENTS

XXVIth Annual Colloquium Protides of the Biological Fluids is due to be held in Brugge Belgium May 1–5 1978

Topics Immune complexes Cytoskeletal proteins

Secretariat XXVIth Colloquium—Protides of the Biological Fluids Simon Stevin Instituut Ziekenhuis Sint Jan Ruddershove B 8000 Brugge Belgium

9th International Symposium on Chromatography and Electrophoresis will be held at Riva del Garda Lake of Garda Italy May 15–17 1978

Organizers the Belgian Society for Pharmaceutical Sciences the Italian Group for Mass Spectrometry in Biochemistry and Medicine and the Italian Society for Pharmaceutical Sciences

Secretariat Dr A Frigerio Istituto di Ricerche Farmacologiche Mario Negri Via Eritrea 62 I 20157 Milan Italy

5th International Symposium on Mass Spectrometry in Biochemistry and Medicine organized by the Italian Group for Mass Spectrometry in Biochemistry and Medi will be held in Rimini Italy June 19–21, 1978

retariat Dr A Frigerio Istituto di Ricerche giche Mario Negri Via Eritrea 62 I 20157 ilan Italy

VIIIth World Congress of Cardiology sponsored by the International Society and Federation of Cardiology organized by the Japanese Circulation Society and the Japan Heart Foundation will take place in Tokyo Japan Sept 17–23 1978 President M Yoshio Secretary General S Hiroshi The Scientific Committee welcomes submission of abstracts on original studies Some papers may be selected for presentation at symposia Abstracts should reach the Congress Secretariat by March 31 1978

Secretariat 7 3 23 Roppongi Minato ku Tokyo 106 Japan

XVIIth Czechoslovak Congress with International Participation on Gastroenterology organized by the Slovak Gastroenterological Society and the Czechoslovak Hepatological Society commissioned by the Czechoslovak Medical Society J E Purkyně will take place in Bratislava Czechoslovakia *Sept 18–20 1978 Chairman* M Brix Secretary General M Lukáč

Topics Esophagus and stomach Intestinal tract Hepatobiliar system and pancreas Examination methods in gastroenterology Free papers (summaries are to be submitted by April 15 1978)

Secretariat Congress Office, Slovak Medical Society Mickiewivczova 18 883 22 Bratislava Czechoslovakia

3rd European Congress on Ultrasonics in Medicine will be held in Bologna Italy Oct 1–5 1978 Official Language English

Scientific secretariat Prof C Alvisi Clinica Neurochirurgica dell Università Via Ugo Foscolo 7 I-40123 Bologna Italy

Organizing secretariat Centro Minerva Medica Via L Spallanzani 9/11 I-00160 Rome Italy

Symposium with International Participation on Exercise and Cardiovascular Function organized by the Czechoslovak Society for Rehabilitation and the Czechoslovak Society of Cardiology under the protection of the Rehabilitation Council of the International Cardiological Society will be held in Bratislava Czechoslovakia Oct 11–13 1978

Topics Influence of exercise on circulation Exercise in the rehabilitation programmes in cardiovascular diseases Free communications

Information M Palát MD Dept of Physical Medicine and Rehabilitation Derer s Hospital Limbova ul 5 809 46 Bratislava Kramáre Czechoslovakia and Slovak *Medical Society Congress Office Mickiewivczova 18* 883 22 Bratislava Czechoslovakia

XIVth International Congress of Internal Medicine will be held in Rome Italy Oct 15–19 1978

Further information and registration forms Secretary General of the Congress Prof M Sangiorgi I Clin ca Medica Policlinico Umberto I I-00161 Rome Italy

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EDITORIAL

The study of signals indicating cancer has become one of the most important chapters of modern oncology These signals may either be purely qualitative when it is known that their presence announces the growth of some special malignant neoplasm in the body Or their study may give more or less quantitative results when we find that decrease or increase runs parallel with changes in the tumour load Disappearance of the signal together with eradication of the tumour is a proof that a causal relationship exists i e the mechanism is truly paraneoplastic it represents an influence from the tumour on the body mediated by some tumour products

Most conspicuous among the cancer signals is a dermatological symptom easily visible on the body surface of the patient In this number of the journal we publish a colour plate picturing rather striking symptoms from the skin that all disappeared during effective treatment of the neoplasia The connections between the malignant cell and the epidermis are a mystery but as often in clinical medicine we have first to rely on pure empiricism and hope that we shall later gain access to deeper causes

We have also tried to collect a series of papers on myeloma and related diseases treating paraneoplastic conditions of other kind caused by the products of the malignant cell Hyperviscosity cold agglutinin symptoms from skin and blood cryoprecipitation of macroglobulins and other Ig molecules are wellknown mechanisms of disease Another result of the presence of an abnormal protein is analyzed in a paper on hyponatraemia in myeloma patients who produce a cationic immunoglobulin This is certainly an interesting phenomenon that deserves further study An illustration of the complexity in the symptomatology of some patients who definitely suffer from a plasma cell dyscrasia to use a term coined by Osserman is a syndrome that was first studied in Scandinavia and England Osteosclerotic skeletal changes with focal lysis moderate infiltration of the bone marrow with plasma cells as well as very moderate increase in one monoclonal Ig with polyneuropathy as the dominant clinical symptoms seems to be a pretty complex and seemingly incoherent flora of symptoms Recent Japanese studies have disclosed that the syndrome may be much more complicated with gynaecomastia in males hirsuties glucosuria hypertrichosis and disturbances of hormone metabolism The relation of all these (paraneoplastic?) symptoms to the presumed myeloma is difficult to grasp

Complications of another kind from the nervous system are of course common and caused by mechanical pressure Unander Scharin and collaborators have analyzed the reults of surgery both in paraplegic myeloma patients and as an attempt to strengthen bones in the limbs that are on the verge of fracturing The clinical picture of myeloma has changed completely after the advent of Melphalan and cyclophosphamide This makes it imperative to help the patients to get a better life and not only a longer Even when treatment is effective in many ways it is a fact of great concern that fractures still occur and that healing of lytic bone lesions is exceedingly rare More experience is needed to decide which is the best treatment laminectomy + tumour extirpation irradiation chemotherapy or preferably all the three together It is a truism to say that improved therapeutic results create new problems and complicate clinical decision making This is true of almost any facet of tumour therapy be it surgical chemical or hormonal

There has been much discussion regarding toxicity of different alkylating agents used in the treatment of myeloma Many reports state that cyclophosphamide should be specially friendly to the platelets but convincing proof of this statement has been difficult to find A short study in this number of the journal seems to give a clearcut answer in the affirmative A comparison of the Melphalan and the cyclophosphamide treated groups showed that the decrease in leucocytes was the same in both whereas the decrease in platelet levels was significantly less in the latter group The explanation for these differences in sensitivity regarding the megakaryocytes is not easy to find As a basis for therapy this is important however severe thrombocytopenia is a life threatening situation

The central problem in the discussion of the

monoclonal gammapathies is of course the etiology Why should one clone of plasma cells or lymphocytes proliferate and secrete one individual immunoglobulin molecule at the same rate during decades when the condition remains benign? Malignant transformation is accompanied by rapidly and cotinuously increasing levels In this number Finnish authors have analyzed their study of persons with monoclonal Ig bands on electrophoresis The follow up time has not always been long and in view of the fact that time is so far the only factor that differentiates between static benign and progressive malignant long time observation is all important We have now in Malmo collected a large number of case histories of persons followed for more than four years up to 18 years The levels keep surprisingly constant both for IgG IgA and IgM The monoclonal gammaglobulin never disappears except in a few persons who always have had very low levels The macroglobulinaemic persons may have a slow but steady increase without developing other symptoms

his situation is very different from antibody tion after challenge with an antigen Immuno who are used to regard antibody formation a response to a specific stimulus would like to assume that a chronic stimulus (infection?) is present Osserman has pointed to the fact that chronic infections of the gallbladder and colon are common in these persons He has also stressed the point that the colon contains a large store of bacterial antigens that might well start production of antibodies On the other hand the large majority of persons with such illnesses do not have any production of monoclonal Ig in high quantities In 1968 I wrote a paper Antibodies without antigenic stimulation and I am still of the opinion that a random derepression of one polypeptide forming template may be a parallel of the same kind as we see it in other tumours as a paraneoplastic phenomenon This mechanism will certainly be studied in a great many clinical conditions in the future In the paper from Finland the authors have listed all other diagnoses especially neoplasms that their patients with monoclonal gammapathies have had This is in some ways comparable to the making of lists containing complicating diseases of patients with gastric ulcer diabetes or myocardial infarction A great many persons especially in the higher age brackets suffer from several although unrelated diseases This is especially true of the persons with the benign disturbances of the gammaglobulin pattern They live very long and therefore have time to develop all sorts of diseases The only proof of a truly paraneoplastic condition is the reversibility when the primary disease is healed This has never been shown not even in persons who have monoclonal gammapathy and a carcinoma that may be radically resected Clinical observations along these lines are very important however

Another way to attack this problem that has great importance for oncology (antibody formation against cancer cells!) is the statistical We are at present collecting data on all the macroglobulinaemic persons from Malmo during a long period of time and try to compare these data with the normal cancer incidence in Malmo that is very well known I am not convinced, however that the results will be decisive

The problem regarding the localization of the cells that produce monoclonal Ig needs continued investigation The Dutch group working with Hijmans and v Furth could show that even when the percentage of the plasma cells in the bone marrow was not increased the percentage of cells producing the type of Ig that was present in the monoclonal spike was quite high Turesson has extended these observations on a large group of well controlled patients He found that the bone marrow of all the 85 persons with monoclonal gammapathy investigated with group specific (H and L chain) immunofluorescent antisera cotained cells with predominance of the heavy and light chain group that was found in the serum M component This was true both for myeloma for benign gammapathy and for lymphomas Such cells occurred in increased amount in extramedullary lymphatic tissues from myeloma patients but not in the other two groups The author points out that this indicates that cells producing an M component are primarily located in the bone marrow with limited extramedullary spread With higher tumour load metastases occur in other organs The homing of Ig producing cells to the red bone marrow is beautifully illustrated by these findings Also in lymphoma patients the clone that produced monoclonal Ig could be traced to the bone marrow even when histology of this organ gave no indication of lymphoma Studies on the synthetic rate of Ig in these conditions were performed and the reader should consult the original paper The depression of other Ig forming clones than the dominating is also discussed

The editor hopes that the publication of an Acta Medica number chiefly treating related subjects will become popular among the readers We shall try to find guest editors who may be willing to edit special numbers on other topics It is my feeling that in the near future oncology will become amalgamated with internal medicine This means that paraneoplasia will have a central position The editor has just completed a monograph on this subject that will be published shortly

Jan G Waldenstrom

BOOK REVIEW

Progress in cancer research and therapy vol 3 Genetics of human cancer Edited by John J Mulvihill Robert W Miller and Joseph F Fraumeni, Jr 519 pages Sw cr 170 Raven Press New York 1976

The title genetics of human cancer will certainly persuade many biologically minded physicians and of course a large number of basic scientists to read the book During recent years it has become clear that the connection between the biochemistry of the genetic material and the problem of cancer is very close

In this volume a number of top ranking experts have published their opinions on fundamental topics As the volume is based on the proceedings of a conference sponsored by the National Cancer Institute in Bethesda there is also a report—often very stimulating—of the discussion after the papers

It is impossible to give a short synopsis of the contents Names such as Melvin Block Fraumeni Robert Miller and Terasaki are well known to clinicians all over the world From a clinical point of view I would regard the papers by the eminent radiobiologist Cleaver and the clini an Fialkow as the most fascinating Cleaver was the first on who could show that a liability to develop cancer inherited in the way that is characteristic for inborn ors of metabolism that is a genetically determined enzyme defect These studies on the rare skin disease xeroderma pigmentosum have been extended and it is now clear that different enzymes all necessary for the repair of broken strands of DNA may cause different types of the same clinical disease The proof that different enzymes may be responsible in different families has been given in a very elegant type of experiment with fusion of cells If fibroblasts from an affected member of one family are fused with cells from another patient in the same family the biochemical error is not abolished as it is by fusion of diseased with normal fibroblasts By collecting members of different families with the disease Cleaver could show that some cell combinations from two patients in different families became normal

This can only be explained by assuming that in one family an enzyme A is defective in another B A+A does not give a normal enzyme pattern whereas A+B abolishes the defect This illustrates the correctness of the hypothesis that the same clinical picture—the same phenotype—may be caused by different mutations These models for the understanding of the molecular basis for inherited diseases are so far unique but certainly have wide application in medicine

Fialkow who is professor of medicine in Seattle has written an interesting chapter on a subject where he has done outstanding and pioneering work Clonal origin and stem cell constitution of human tumours Ever since Lyons made her fundamental assumption that in the female organism either one of the two X chromosomes in the cell is repressed (inactive) it was clear that female heterozygotes for a character (A) that can be defined on the cellular level must have two types of cells one containing X^A the other normal X Such genetic markers are very rare and the only one that has become important for genetic work is mutations of the DNA for the G 6-PD molecule Fialkow has now studied a large number of female carriers of such mutated genes and could show that the cellular populations in chronic myeloid leukemia must come from stem cells belonging only to one type i e who are monoclonal Fibroblasts from such persons are biclonal Also in polycythemia vera a monoclonal proliferation has been established but we have reason to believe that a small part of normal cells is also present Fialkow also discusses the situation in multiple myeloma lymphatic leukemia and Waldenstrom s macroglobulinemia where biochemical analysis of the produced immunoglobulins has introduced the concept of monoclonality

For solid tumours the problem is much more difficult to solve Most results seem to indicate that their origin is multicellular

Interesting chapters on cytogenetics of human neoplasia chromosome instability syndromes the connection between certain karyotypic changes etiology of tumours and genetic markers in cancer should find readers among different specialist groups in the medical profession It is quite remarkable and somewhat depressing to find that the many studies of familial cancers have contributed so little to our deeper understanding of the cancer problem

The book is expensive and it will only have the freshness of actuality for a limited period Still it must be strongly recommended to all who have an active interest in the fundamental problems of carcinogenesis The only flaw is the remarkably poor quality of the reproduced photographs

Jan G Waldenström

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Complete Reversibility of Paraneoplastic Acanthosis Nigricans after Operation

H Moller Sten Eriksson O Holen and J G Waldenstrom

From the Departments of Dermatology Internal Medicine and Surgery University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT A patient with widespread acanthosis nigricans is described No abdominal tumour was found Explorative thoracotomy disclosed numerous enlarged lymph glands containing squamous cell carcinoma The left lung was removed but meticulous search did not disclose any tumour The glands were removed as radically as possible After the operation the skin improved and the lesions have disappeared completely The observation time is over three years

One of the most enigmatic among the paraneoplastic symptoms from the skin is acanthosis nigricans (AN) Nothing important is known about the mechanism behind the striking changes in the skin and the literature contains surprisingly few if any really convincing examples of reversibility after successful operation with a long term follow up We have observed one such patient and think that this may be worth a short report

CASE REPORT

Man born in 1914 (Plate Fig e) The patient has for a long time smoked 12–15 cigarettes per day Previous maladies are of no special interest In March 1972 he noted the appearance of numerous small warts on both arms neck back and solitary in the face and on the medial part of the thighs About the same time he noticed that the skin on the neck armpits and groins became very dark

He was admitted to the Department of Dermatology Malmo General Hospital in May 1972 There was a symmetric ashy gray hyperpigmentation of the neck armpits nipples and groins The pigmented skin was made up of a papillomatous hyperkeratosis There was a large amount of skin-coloured verrucous tumours particularly in the armpits and groins but also on the dorsa of the hands the shoulders and the face In the palms and soles there was a diffuse yellowish hyperkeratosis The lips tongue and oral mucosa were hyperplastic and fissuring the hard palate red and granular A biopsy from the groin showed papillomatosis with melanization of the basal epithelial layer ESR was 11 mm/1 h he had no fever The electrophoretic picture of the serum proteins was normal as were the serum electrolytes No signs of parenchymal liver disease were found On X ray oesophagus stomach duodenum and lungs showed nothing remarkable I v pyelography gave findings possibly indicating a tumour or a cyst on the left side Renal angiography showed one cyst on the left side and one on the right Bronchoscopy and cytology of the sputum gave normal results As his external appearance looked acromegaloid the sella turcica was investigated and found to be normal on X ray Somatotropin was normal In Aug 1972 further examinations in order to detect a hidden carcinoma were performed but gastroscopy with biopsy as well as arterial coeliacography and repeated X ray of colon and stomach gave only normal findings In Sept 1972 considerable progression of the skin symptoms was noted with an increasing number of papules In Aug and Dec of that year the skin symptoms deteriorated further He was much worried by the fact that people stared at him in the street On the last occasion an examination with a gastrocamera was performed with negative results

In Aug 1974 the patient was admitted to the Department of Medicine He now had very marked itching all over the body and his skin had become much more heavily pigmented His appetite had remained good There was no weight loss He had no difficulties with micturition but had noted some cough in the morning which did not really bother him Only the skin showed increasing symptoms A new bronchoscopy indicated a narrowing of the bronchial ostium to the lingula that looked somewhat suspect but biopsy showed nothing malignant. Cytology of the bronchial secretions gave no definite information After much discussion with the pathologists and the thoracic surgeons yet another bronchoscopy was performed in Aug 1974 ESR had now increased to 34 mm/1 h He had slight anemia α fetoprotein and the electrophoretic picture were normal

The patient was admitted to the Department of Thoracic Surgery for operative treatment if this seemed to be indicated An exploratory left thoracotomy was performed on Sept 23 1974 Very numerous enlarged lymph glands were found in the hilus and mediastinum but on operation no tumour was detected in the lung. A complete pulmonectomy was performed Very careful exami

nation of the lung after exurpation showed no signs of a tumour The lymph glands were infiltrated by a poorly differentiated squamous cell carcinoma

Around the main bronchus there were several lymph glands the size of Brazil nuts Very careful dissection of the lung only disclosed enlarged glands of the same macroscopical appearance Also mediastinal glands were involved The tumour tissue in the glands contained necroses the cells have a light eosinophilic cytoplasm the nuclei are rather polymorphous and contain nucleoli The cells are partly squamous possibly there are some foci showing keratinization Many microscopical slides from lung tissue were prepared They did not show any signs of carcinoma

The postoperative course was rather stormy but the patient could be sent home with a tracheal cannula. He has been followed regularly during the last three years His skin is completely healed and has remained normal No clinical signs of relapse have been noted

DISCUSSION

The literature on AN is quite extensive The clinical picture in our patient is classical and characteristic for the condition and need not be repeated It should perhaps be mentioned that AN has been en in very obese persons

The most important practical consequence when finding AN is the absolute necessity to use all diagnostic methods in order to find a hidden carcinoma Different authors have collected their own materials together with published cases In one review it was stated that gastric carcinoma was present in over 60% other abdominal cancer in about 25% and non abdominal cancer in 11%

Curth (3) has written extensively on AN She maintains that adenocarcinoma of the GI tract completely dominates the picture and regards the connection with squamous cell carcinoma as not definitely proven It is of course usually correct to say that only complete examination post mortem can exclude the presence of a second carcinoma of adenomatous character In this connection our observation is of interest because it proves that removal of squamous cell carcinoma may cure AN

Regarding the reversibility of the symptoms after removal the literature contains very little positive information Brown and Winkelmann in an excellent review article (2) describe One patient a 34-year-old woman who had adenocarcinoma (grade 2) of the rectosigmoid colon is alive and well 14 years after the resection of the carcinoma A follow up letter in April 1966 indicated that the acanthosis nigricans had regressed after surgery and was no longer evident This patient had obviously not been examined by the authors

Curth et al write in 1962 In many cases in this series regression of the dermatosis after the removal of the carcinoma and spreading of the dermatosis at the time of renewed activity of the tumour has been observed In a more recent paper from 1972 Lerner (4) states regarding prognosis The general impression is that acanthosis nigricans subsides when the associated systemic abnormality is treated adequately be it obesity endocrine disease or malignancy However this general impression has not been substantiated by accurate observation

The causal connections between the tumour and the changes in the skin leading both to hyperkeratosis with acanthosis and verrucae and to very dark pigmentation are quite enigmatic It has been known for a very long time that a high percentage of elderly dachs hounds get symptoms very similar to AN Bornfors (1) studied these animals very carefully and tried to influence the skin symptoms in many different ways He found that thyroidectomy either surgical or with thiouracil clearly improved the status Most striking was the positive effect of TSH (thyroid stimulating hormone) It is also interesting that a very similar if not identical skin picture may occur in young diabetic females with the type of disease where fat is completely lacking (lipodystrophy) These patients may show increased growth and a connection with somatotropin has been assumed These girls have no tumours and the combination of symptoms indicates hormonal influences AN has been described also in obese patients

It seems clear that some substance produced by the tumour must be responsible for the development of AN Many facts speak in favour of the assumption that we should look for an active polypeptide The presence of TSH should perhaps be investigated

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Fg a Pyoderma gangraenosum from patient E L

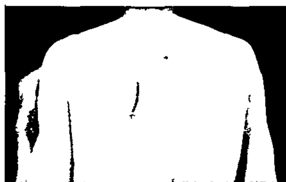
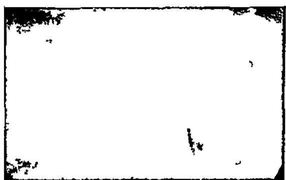


Fig b Erythema annulare centrifugum from pat ent G A



Fg c and d Erythema from patient E H



Fg d



Fg e and f Acanthos s n gr cans from patients described n the text



Fg f

Table I *Age sex and M component heavy and light chain type in 39 patients with myeloma, 36 with benign monoclonal gammapathy (BMG) and 10 with lymphoma*

BMG group includes one patient with Gaucher s disease one with auto-immune hemolytic anemia and two with primary generalized amyloidosis

Patient group	Age (y) Mean	Range	Male/ female	M component heavy and light chain AK	AL	MK	ML	GK	GL	K	L
Myeloma	64	38–90	21/18	4	4	0	2	10	6	11[a]	2
BMG	69	42–84	17/19	5	4	3	0	9	13	2[b]	0
Lymphoma	61	53–85	7/3	0	0	3[c]	3	5	0	0	0

[a] In two cases M component classification from immunofluorescence only
[b] In one case M component classification from immunofluorescence only
[c] One case with biclonal gammapathy (MK GK)

or monoclonal light chains in urine and subnormal serum level of at least one Ig class 2) ≥10% plasma cells in a bone marrow smear 3) Osteolytic lesions demonstrated by X ray examination and without any other obvious explanation or macroscopic bone lesions at autopsy where microscopy revealed proliferation of plasma cells Thirty seven patients fulfilled criterion 1 and at least one of criteria 2 and 3 and were classified as having multiple myeloma Two patients who fulfilled criteria 2 and 3 t not 1 were also included in the myeloma group

hirty patients were classified as having BMG according o the following criteria 1) An M component in plasma th or without small amounts of monoclonal light chains in urine 2) No oesteolytic lesions at X ray examination and/or no macroscopic bone lesions at autopsy 3) No change or very slow progress of M component concentration (i e maximal increase 2 g/l per year) during an observation time of at least two years The mean observation time was 69 months (Table III) Five patients whose M component concentration was followed for less than two years were also included namely three patients who died within two years and in whom autopsy showed no evidence of myeloma one patient with cold agglutinin hemolytic anemia for 15 years and one patient with an observation time of less than one year who had primary generalized amyloidosis no osteolytic lesions and only a slightly increased number of plasma cells in the bone marrow The latter patient had no detectable M component in serum or monoclonal light chains in urine but was considered to have monoclonal gammapathy on the basis of the immunofluorescent demonstration of dominance of kappa chain producing cells in the bone marrow One patient had no detectable serum M component but a constant level of monoclonal kappa chains in urine for 33 months Four of the patients in the BMG group had diseases known to be associated with M components cold agglutinin hemolytic anemia (1 patient) Gaucher s disease (1 patient) and primary generalized amyloidosis (2 patients)

Ten patients had malignant lymphoma The diagnosis was based on lymph node and/or bone marrow biopsy but complete evaluation including staging was not performed in all cases One of the patients had Hodgkin s disease and the others non Hodgkin lymphomas diffuse well differentiated lymphocytic lymphoma (6 patients) nodular histiocytic lymphocytic lymphoma (1 patient) and diffuse histiocytic lymphoma (2 patients) None had the full blown picture of Waldenstrom s macroglobulinemia

Bone marrow was aspirated from the sternum and/or the iliac crest in 84 of the 85 patients Splenic tissue was obtained at autopsy in 9 cases (5 myeloma 4 BMG) and by splenectomy in 2 cases (1 myeloma 1 BMG) Peripheral lymph nodes were biopsied in two cases of lymphoma and a mesenterial lymph node was obtained at autopsy in one case of myeloma Serum was taken from the patients on the same day as or sometimes within

Table II *Serum M component concentration (g/l) in all patients with monoclonal gammapathy excluding those with only Bence Jones proteinuria*

Patient group	IgA N	Serum conc Mean	Range	IgM N	Serum conc Mean	Range	IgG N	Serum conc Mean	Range
Myeloma	8	40 8	7 0–128 0	2	35 4	16 0–54 8	16	50 2	26 0–86 0
BMG	9	13 4	8 0–19 0	3	10 5	6 0–15 2	22	14 8	2 0–58 0
Lymphoma	0	–	–	6	12 0	5 0–22 0	5	19 0	6 0–44 0

2-3 days of the bone marrow aspiration Thirteen of the myeloma patients had been treated with cytostatics before the bone marrow examination

METHODS

Immunofluorescence

Single cell suspensions were prepared from the aspirated bone marrow splenic or lymph node tissue The cells were spread on cytocentrifuge slides fixed and stained with FITC (fluorescein isothiocyanate) or TRITC (tetra methyl isothiocyanate) conjugated antisera against human immunoglobulin heavy chains alpha mu and gamma (Nordic Immunological Laboratories Tilburg The Netherlands) and kappa and lambda light chains (Dakopatts Copenhagen Denmark and gift from Dr W Hijmans Rijswijk The Netherlands) The details of the technique have been reported earlier (8 20) In each case one slide was Pappenheim stained Sections of spleens and lymph nodes were stained with hematoxylin and eosin

The slides were examined with a Leitz Orthoplane fluorescence microscope with an HBO 100 mercury lamp as the light source and epi illumination and filter systems for the two wave length method (7) The number of cells showing cytoplasmic fluorescence in each slide was determined When the number of fluorescent cells was high only 5-10 central rows in each slide were scored On the basis of counting a minimum of 2 000 fluorescent cells in the five slides the following calculations were made the percentage of Ig-containing cells (IgCC) positive for IgA IgM or IgG respectively the ratio kappa positive/lambda positive cells (k/l ratio) and the ratio cells positive for heavy chains/cells positive for light chains (H/L ratio) The nucleated cells were counted in one central row of the Pappenheim stained slide and the fluorescent cells in one row of the slide containing the highest number of positive cells From these figures the following calculations were made the frequency of cells positive for IgA IgM IgG kappa or lambda expressed as cells per 1 000 of all nucleated cells in the preparation

The reliability of the calculations was checked by random duplicate counting of nucleated cells and fluorescent cells in pairs of slides from one cytocentrifugation

Immunoglobulins

M-components were detected by agarose gel electrophoresis (10) and the heavy and light chain types identified by classical immunoelectrophoresis Serum concentrations of IgA IgM and IgG were determined by electroimmunoassay (4 11) In the case of M-components of the IgG class the background level of polyclonal IgG was estimated by visual inspection of the agarose gel and subtracted from the total IgG concentration to obtain the M-component concentration Immunoglobulin kappa and lambda light chain concentrations were measured in unconcentrated urine by single radial immunodiffusion (12) In all cases where no M-component was detected in serum the urine was examined by agarose gel electrophoresis and immunoelectrophoresis If the results were negative the examinations were usually repeated on urine that had been concentrated 50-200 times The examinations were performed by Dr A O Grubb at the Department of Clinical Chemistry

Table III *Observation time and change in M component concentration in 36 cases of BMG*

Observation time (y)	N	Maximal change in M component concentration (g/l)	
		Decrease	Increase
>10	5	9	4
5-10	9	8	
2-5	17	6	5
<2	5	7	4

RESULTS

Fig 1*a* presents the percentages of Ig containing bone marrow cells positive for IgA in patients with an M component of IgA type For comparison the figure also shows the percentages of IgA containing cells in 28 patients without an M component These cases which include several patients with a selective polyclonal increase in one Ig class have been presented earlier (20) All patients with an M component of IgA type have a higher percentage of IgCC positive for IgA than any of the patients without an M component The dominance of IgA containing cells is more pronounced in the myeloma group than in the BMG group but there is considerable overlapping Figs 1*b* and 1*c* show the corresponding values for IgM and IgG-containing cells in patients with an M component of IgM or IgG type respectively Again there is a dominance of IgCC positive for the same heavy chain class as that of the M component whether it represents myeloma BMG or lymphoma There is however some overlapping with individuals without an M component The k/l ratios of Ig containing bone marrow cells in all patients with an M-component in serum and/or monoclonal light chains in urine are compared with the k/l ratios of 26 patients without an M component in Fig 1*d* All patients in the former group could be distinguished by higher or lower k/l ratios than the patients without an M component with overlapping in only one case

In 5 cases of myeloma 12 cases of BMG and 2 cases of lymphoma bone marrow was aspirated at the same time from different localizations The frequency of IgCC and their distribution by Ig classes was calculated There is a close similarity

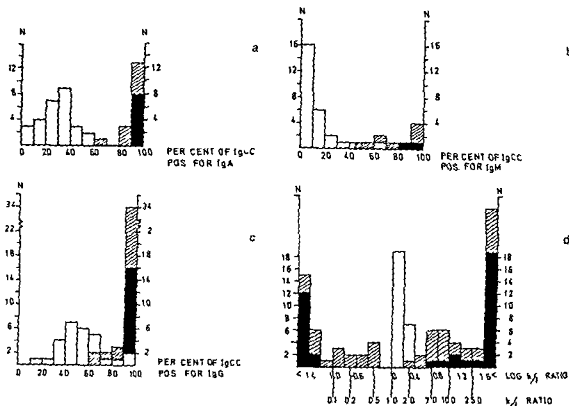


Fig 1 Percentage distribution by Ig classes and kappa/λ ratio (k/l ratio) of Ig containing bone marrow cells (IgCC) in patients without an M component and in patients with monoclonal gammapathy (a) IgA M components (b) IgM M components (c) IgG M components (d) all cases of monoclonal gammapathy □=No M component ▨=monoclonal gammapathy including myeloma ■=myeloma

in both these respects between the bone marrow samples from two or three different sites in one and the same individual (Table IV)

The frequency of IgCC and their distribution by Ig classes in spleen and lymph nodes from patients with monoclonal gammapathy are presented in Table V They are compared with the corresponding figures for bone marrow taken in most cases on the same occasion at autopsy or in some cases by aspiration before an operation In one case of myeloma no IgCC could be detected in the spleen In all other myeloma cases the majority of IgCC in the spleen and, when examined lymph node were positive for the same class of heavy and light chain as forming the M component In contrast in 4 of 5 cases of BMG and 1 of 2 cases of lymphoma the class distribution and k/l ratio of IgCC indicated a polyclonal proliferation in the spleen or lymph node but monoclonal proliferation in the bone marrow (the bone marrow was not examined in one case) In those cases where the monoclonal distribution was present also in the spleen or lymph node the frequency of IgCC was usually much higher in the bone marrow

Three patients with lymphoma are of special interest They showed no routine histologic evidence of bone marrow involvement of the lymphoma but the immunofluorescence staining of the bone marrow cells indicated a monoclonal proliferation of cells producing the same class of heavy and light chain as forming the M component (MK GK and GK respectively) The lymphoma tissue was examined by immunofluorescence in one of them A biopsy of an involved lymph node revealed a nodular mixed histiocytic lymphocytic lymphoma but the majority of the cells were not positive for intracellular Ig and those that were positive showed a polyclonal distribution

The number of IgCC in the bone marrow was compared with the product of a constant representing the plasma volume and the fractional catabolic rate (FCR) and serum level of each Ig class FCR was not determined in each individual case but mean values were taken from the literature The

Table IV *Distribution of Ig containing cells (IgCC) by Ig classes and number of IgCC positive for IgA IgM or IgG per 1000 nucleated bone marrow cells Comparison between bone marrow from sternum (STP) iliac crest (CRP) and spinal process (SPP)*

k/l=kappa/lambda ratio H/L=heavy chain/light chain ratio

	Ig class distribution of IgCC					IgCC per 1 000 nucleated cells		
Source of cells	IgA (%)	IgM (%)	IgG (%)	k/l	H/L	IgA	IgM	IgG
Myeloma cases								
STP	2	1	97	50 9	1 0	1 0	0 4	55 6
CRP	2	<1	98	34 0	1 0	1 2	0 1	62 0
STP	2	1	97	0 02	–	–	–	–
CRP	3	1	96	0 05	–	–	–	–
SPP	3	1	96	0 05	–	–	–	–
STP	<1	<1	>99	100	1 0	–	–	–
CRP	<1	<1	>99	100	1 0	–	–	–
CRP	20	11	69	100	0 01	–	–	–
SPP	17	12	71	100	0 01	–	–	–
STP	4	86	10	0 07	1 0	1 3	27 4	3 2
CRP	3	92	5	0 05	0 9	1 1	38 8	2 1
BMG cases								
STP	96	<1	4	46 0	1 1	79 8	0 1	3 3
CRP	97	<1	3	64 3	1 0	111 2	0 3	3 4
STP	81	1	18	10 1	1 1	30 1	0 2	6 6
CRP	85	1	14	11 3	1 1	26 7	0 1	4 5
STP	68	2	30	0 2	1 3	–	–	–
CRP	68	2	30	0 1	1 3	–	–	–
STP	94	<1	6	0 05	1 1	50 1	0 1	3 4
CRP	95	<1	5	0 03	1 0	44 6	0 1	2 2
STP	93	<1	7	0 08	1 0	82 5	0 5	6 0
CRP	93	<1	7	0 08	1 0	98 7	0 3	7 1
STP	7	81	12	6 0	1 2	–	–	–
CRP	6	71	23	9 0	1 4	–	–	–
STP	<1	98	2	100	–	0 5	162 9	2 6
CRP	<1	98	2	100	–	0 2	159 2	1 3
STP	3	<1	97	0 07	1 1	3 0	0 4	111 1
CRP	3	<1	97	0 04	1 1	3 0	0 4	113 5
STP	3	<1	97	0 04	1 1	2 7	0 2	93 6
CRP	4	1	95	0 05	0 8	3 2	0 3	72 7
STP	30	8	62	3 5	1 2	–	–	–
CRP	19	2	79	2 6	–	–	–	–
SPP	13	3	84	4 6	1 1	–	–	–
STP	7	2	91	0 19	–	8 1	2 0	103 3
CRP	5	1	94	0 10	1 3	5 7	1 3	113 3
STP	20	1	79	5 7	1 0	9 3	0 2	36 5
CRP	15	1	84	5 1	1 0	3 6	0 3	19 7
Lymphoma cases								
STP	18	53	29	0 52	1 1	3 4	10 2	5 6
CRP	18	54	28	0 47	1 4	4 4	12 9	6 5
STP	9	3	88	4 7	1 5	–	–	–
CRP	[illegible]	2	96	13 1	1 5	2 5	1 0	43 7

–=Not done

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Table IV *Distribution of Ig containing cells (IgCC) by Ig classes and number of IgCC positive for IgA IgM or IgG per 1000 nucleated bone marrow cells Comparison between bone marrow from sternum (STP) iliac crest (CRP) and spinal process (SPP)*

k/l kappa/lambda ratio H/L heavy chain/light chain ratio

Source of cells	Ig class distribution of IgCC					IgCC per 1 000 nucleated cells		
	IgA (%)	IgM (%)	IgG (%)	k/l	H/L	IgA	IgM	IgG
Myeloma cases								
STP	2	1	97	50 9	1 0	1 0	0 4	55 6
CRP	2	<1	98	34 0	1 0	1 [illegible]	0 1	62 0
STP	2	1	97	0 07				
CRP	3	1	96	0 05				
SPP	3	1	96	0 05				
STP	<1	<1	>99	100	1 0			
CRP	<1	<1	>99	100	1 0			
CRP	20	11	69	100	0 01			
SPP	17	12	71	100	0 01			
STP	4	86	10	0 07	1 0	1 3	27 4	3 2
CRP	3	92	5	0 05	0 9	1 1	38 8	2 1
BMG cases								
STP	96	<1	4	46 0	1 1	79 8	0 1	3 3
CRP	97	<1	3	64 3	1 0	111 2	0 3	3 4
STP	81	1	18	10 1	1 1	30 1	0 2	6 6
CRP	85	1	14	11 3	1 1	26 7	0 1	4 5
STP	68	2	30	0 2	1 3			
CRP	68	2	30	0 1	1 3			
STP	94	<1	6	0 05	1 1	50 1	0 1	3 4
CRP	95	<1	5	0 03	1 0	44 6	0 1	2 2
STP	93	<1	7	0 08	1 0	82 5	0 5	6 0
CRP	93	<1	7	0 08	1 0	98 7	0 3	7 1
STP	7	81	12	6 0	1 2			
CRP	6	71	23	9 0	1 4			
STP	<1	98	2	100		0 5	162 9	2 6
CRP	<1	98	2	100		0 2	159 2	1 3
STP	3	<1	97	0 07	1 1	3 0	0 4	111 1
CRP	3	<1	97	0 04	1 1	3 0	0 4	113 5
STP	3	<1	97	0 04	1 1	2 7	0 2	93 6
CRP	4	1	95	0 05	0 8	3 2	0 3	72 7
STP	30	8	62	3 5	1 2			
CRP	19	2	79	2 6				
SPP	13	3	84	4 6	1 1			
STP	7	2	91	0 19		8 1	2 0	103 3
CRP	5	1	94	0 10	1 3	5 7	1 3	113 3
STP	20	1	79	5 7	1 0	9 3	0 2	36 5
CRP	15	1	84	5 1	1 0	3 6	0 3	19 7
Lymphoma cases								
STP	18	53	29	0 52	1 1	3 4	10 2	5 6
CRP	18	54	28	0 47	1 4	4 4	12 9	6 5
STP	9	3	88	4 7	1 5			
CRP	2	2	96	13 1	1 5	2 5	1 0	43 7

=Not done

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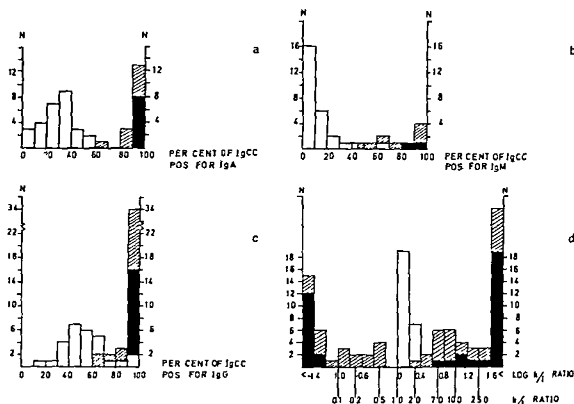


Fig 1 Percentage distribution by Ig classes and kappa/lambda ratio (*k/l ratio*) of Ig containing bone marrow cells (*IgCC*) in patients without an M-component and in patients with monoclonal gammapathy (*a*) IgA M-components (*b*) IgM M components (*c*) IgG M-components (*d*) all cases of monoclonal gammapathy □=No M component ▨=monoclonal gammapathy including myeloma ■=myeloma

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The number of IgCC in the bone marrow was compared with the product of a constant representing the plasma volume and the fractional catabolic rate (FCR) and serum level of each Ig class FCR was not determined in each individual case but mean values were taken from the literature The

Table IV *D str b t o i f Ig co ta g cells (IgCC) b Ig l ss s a d l er f IgCC posit e f r IgA IgM or IgG per 1000 n icleated bone arro cells C j r o bet ee i bo e rro fro i sterm i (STP) l ac crest (CRP) a d sp nal process (SPP)*

k/l kappa/lambda ra o H/L hea y cha n/l ght chain rat o

	Ig class d stribut on of IgCC					IgCC per 1 000 nucleated cells		
Sou ce of ce s	IgA (%)	IgM (%)	IgG (%)	k/l	H L	gA	IgM	IgG
M eloma cases								
STP	2	1	97	50 9	0	0	0 4	55 6
CRP	2	<1	98	34 0			0 1	6 0
STP	2	1	97	0 0				
CRP	3	1	96	0 05				
SPP	3	1	96	0 05				
STP	<1	<1	>99	100	1 0			
CRP	<1	<1	>99	100	0			
CRP	0	11	69	100	0 01		–	
SPP	17	12	71	100	0 01			
STP	4	86	10	0 07	0	1 3	27 4	3 2
CRP	3	92	5	0 05	0 9	1 1	38 8	2 1
BMG cases								
STP	96	<1	4	46 0	1 1	79 8	0 1	3 3
CRP	97	<1	3	64 3	1 0	111 ?	0 3	3 4
STP	81	1	18	10 1	1	30 1	0 2	6 6
CRP	85	1	14	11 3	1 1	6 7	0 1	4 5
STP	68	2	30	0 2	1 3			
CRP	68		30	0 1	1 3			
STP	94	<1	6	0 05	1	0 1	0 1	3 4
CRP	95	<1	5	0 03	1 0	44 6	0 1	2 2
STP	93	<1	7	0 08	1 0	82 5	0 5	6 0
CRP	93	<1	7	0 08	1 0	98 7	0 3	7 1
STP	7	81	12	6 0	1 2			
CRP	6	71	23	9 0	4			
STP	<1	98	2	100		0 5	62 9	2 6
CRP	<1	98	2	100		0 2	59 2	1 3
STP	3	<1	97	0 07	1 1	3 0	0 4	111 1
CRP	3	<1	97	0 04	1 1	3 0	0 4	113 5
STP	3	<1	97	0 04	1 1	2 7	0 2	93 6
CRP	4	1	95	0 05	0 8	3 2	0 3	72 7
STP	30	8	62	3 5	1 2			–
CRP	19	2	79	2 6				–
SPP	13	3	84	4 6	1 1			
STP	7		91	0 19		8 1	2 0	103 3
CRP	5	1	94	0 10	1 3	5 7	1 3	113 3
STP	20	1	79	5 7	1 0	9 3	0 2	36 5
CRP	15	1	84	5 1	1 0	3 6	0 3	19 7
Lymphoma cases								
STP	18	53	29	0 52	1 1	3 4	10 2	5 6
CRP	18	54	28	0 47	1 4	4 4	12 9	6 5
STP	9	3	88	4 7	1 5		–	–
CRP	?	2	96	13 1	1 5	2 5	1 0	43 7

– = Not done

Table V *Distribution of Ig containing cells (IgCC) by Ig classes and number of IgCC positive for the Ig class of the M component per 1 000 nucleated cells Comparison between bone marrow (BM) spleen (Sp) and lymph node (LN)*

k/l=ratio cells positive for kappa chains/cells positive for lambda chains H/L=ratio cells positive for heavy chains/cells positive for light chains

Diagnosis	M com ponent Ig chains	Source of cells	Ig class distribution of IgCC					IgCC pos for Ig class of the M-comp per 1 000 cells
			IgA (%)	IgM (%)	IgG (%)	k/l	H/L	
Myeloma	AL	BM	99	<1	<1	0 003	1 0	248
		Sp	98	<1	2	0 010	1 0	52
Myeloma	AL	BM	99	<1	<1	0 001	0 9	450
		Sp	No positive cells					
Myeloma	GK	BM	<1	<1	99	108 7	1 2	295
		Sp	<1	<1	99	215 8	1 2	26
Myeloma	GL	BM	2	1	97	41 4	1 0	–
		Sp	9	4	87	5 7	–	–
		LN	7	1	92	4 8	1 0	–
Myeloma	GL	BM	<1	<1	99	0 001	1 0	854
		Sp	<1	<1	99	0 060	1 1	127
Myeloma	K	BM	–	–	–	891 4	0 001	976
		Sp	–	–	–	1 000	0 001	114
BMG	AK	BM	93	<1	7	41 3	1 1	42
		Sp	6	2	92	1 5	1 2	0 8
IG	AL	BM	93	<1	7	0 15	1 2	95
		Sp	86	2	12	0 15	0 9	20
	AL	BM	93	<1	7	0 06	1 1	91
		Sp	34	13	53	1 0	1 0	11
ıG	GK	BM	3	<1	97	51 9	1 1	146
		Sp	18	4	78	1 7	1 2	–
BMG	GL	BM	–	–	–	–	–	–
		Sp	43	12	45	1 5	0 9	–
Lymphocytic lymphoma	MK	BM	1	93	6	34 8	1 0	259
		LN	<1	98	2	92 6	1 0	380
Histiocytic lymphocytic lymphoma	GK	BM	8	2	90	8 3	1 0	61
		LN	5	24	71	1 3	1 1	–

–=Not done

Table VI *Mean calculated synthetic rate of the Ig class of the M component (product of serum concentration fractional catabolic rate FCR and a constant representing the plasma volume PV) compared with mean number of Ig containing cells (IgCC) positive for the same Ig class per 1 000 nucleated bone marrow cells Linear regression*

	Serum conc (g/l)×FCR ×PV	IgCC per 1 000 bone marrow cells	*r*	*p*
IgA (*N*=14)	7 28×PV	129	0 79	<0 001
IgM (*N*=8)	1 72×PV	121	0 79	<0 05
IgG (*N*=36)	4 04×PV	167	0 52	<0 01

FCR of IgA and IgM are independent of the serum concentration and have been estimated to be 0 252 and 0 106 respectively (9 18) The FCR of IgG is dependent on the serum concentration and was calculated in each case from the data presented by Waldman (23) Provided a balance exists between daily synthetic and catabolic rate and individual differences in plasma volume and FCR are disregarded the product gives an approximate estimation of the daily synthetic rate of each Ig class A positive correlation was obtained between the calculated synthetic rate of the Ig class of the M component and the number of IgCC positive for the same Ig class per 1 000 nucleated bone marrow cells

(Table VI). When only the myeloma cases were considered, the correlation was weak or absent.

The calculated synthetic rate and serum level of Ig classes other than that of the M component were compared with the number of IgCC positive for the same Ig class per 1 000 nucleated bone marrow cells excluding cells positive for the Ig class of the M component (Tables VII and VIII). The number of IgCC positive for IgA, IgM or IgG at normal serum Ig levels in individuals without an M component are included in Table VIII for comparison. Again a significant positive correlation was obtained between the number of IgCC positive for each Ig class and the calculated synthetic rate of the same Ig class.

Table VII. *Mean calculated synthetic rate of Ig classes other than that of the M component (product of serum concentration, fractional catabolic rate, FCR, and a constant representing the plasma volume, PV) compared with mean number of Ig containing cells (IgCC) positive for the same Ig class per 1 000 nucleated bone marrow cells excluding those positive for the Ig class of the M component. Linear regression*

	Serum conc (g/l)×FCR ×PV	IgCC per 1 000 bone marrow cells	*r*	*p*
IgA (N=55)	0.27×PV	3.4	0.83	<0.001
IgM (N=60)	0.06×PV	0.6	0.42	<0.001
IgG (N=34)	0.51×PV	5.2	0.87	<0.001

DISCUSSION

In all myeloma cases a large majority of Ig containing bone marrow cells were positive for the same class of heavy and/or light chain as forming the M-component. This is in accordance with several previous reports (7, 14, 19) and strongly indicates that these cells are the source of the M component, although biosynthetic studies and the use of anti-idiotypic antisera are needed for confirmation. In benign monoclonal gammapathy the dominance of M component producing cells was not as pronounced as in myeloma but the BMG cases could be distinguished from cases without an M-component by virtue of the distribution profile of Ig-containing bone marrow cells. Similar results have been reported by others (7). The diagnosis of BMG cannot be firmly established without the finding of an unchanged concentration of the M component during an observation time of several years (22). There do exist, however, cases with a very slow increase in M component concentration who do not develop clinical signs of multiple myeloma during many years of observation. It is probable that some benign M component producing clones have reached a plateau level when the M component is detected, while others are very slowly approaching such a level where the tumor burden is still too small to give clinical signs of multiple myeloma. The small changes in M-component concentration during long observation times indicate a high degree of homeostasis of the benign M-component producing cell clones in the present study. They are also apparently widely distributed in the bone marrow.

Although the dominance of M component producing cells in the bone marrow was more pronounced in myeloma than in BMG there was considerable overlapping and the two conditions cannot be distinguished in this way. Another pattern emerges when extramedullary lymphoid tissue is considered. In myeloma the M-component producing cell clone clearly dominated even in the spleen in most cases, while in BMG the distribution profile of IgCC in the spleen was usually polyclonal. When the M-component producing clone could be traced also to the spleen, the number of positive cells per 1000 nucleated cells was always considerably higher in the bone marrow. These data are compatible with the following hypothesis: M component producing cell clones are primarily located in the bone marrow and extramedullary spread is absent or limited. With higher tumor cell numbers, as in the terminal stages of multiple myeloma, a metastatic spread to lymphoid tissues occurs. At autopsy, extramedullary spread can be detected by routine histological examination in 70% of myeloma cases and the histologic picture often indicates true metastases (3).

In all cases of lymphoma with monoclonal gammapathy the M-component producing cell clone could also be traced to the bone marrow. This was also observed when routine histological examination of the bone marrow gave no evidence of lymphoma. Lymph nodes were examined only in two cases. In one of these (diffuse well differentiated lymphocytic lymphoma) a monoclonal pattern was observed in the lymph node as well as in

Table VIII *Mean serum concentration of Ig classes other than that of the M-component and mean number of Ig-containing cells (IgCC) positive for the same Ig class per 1 000 nucleated bone marrow cells excluding those positive for the Ig class of the M-component A comparison with number of IgCC per 1 000 bone marrow cells in individuals without an M-component and with normal serum Ig levels*

	IgA		IgM		IgG	
Patient group	Serum conc (g/l)	IgCC per 1 000 bone marrow cells	Serum conc (g/l)	IgCC per 1 000 bone marrow cells	Serum conc (g/l)	IgCC per 1 000 bone marrow cells
Myeloma	0 6	2 3	0 3	0.5	5 4	3 2
N	24	24	28	28	20	20
BMG	1.3	4 3	0 8	0 7	9 5	6 4
N	23	23	28	28	11	11
No M-component*	1 8	5 8	1 0	0 7	10 0	8 8

* Data obtained from a previous publication (20)

bone marrow In the other (nodular histiocytic lymphocytic lymphoma) the affected lymph node contained a moderate number of plasma cells with a polyclonal distribution while the bone marrow which was not histologically involved by the lym phoma showed a monoclonal distribution of IgCC ment with the M-component These ob- tions can be explained in two ways The M- nent producing clone may not be directly ted to the lymphoma clone Such patients have been reported by others (16) Alternatively the bone marrow might provide a suitable environment for the maturation of the tumor cells to Ig secreting plasma cells Combined cytoplasmic and membrane staining for immunoglobulin chains in such cases are required to explain these observations

An effort was made to estimate the synthetic rates of IgA IgM and IgG The calculations are of course based on several approximations and do not take into account individual differences in *plasma volume and FCR of the M-components* which might in some cases be of IgG subclass 3 known to be catabolized more rapidly (13) The myeloma cells might be unevenly distributed and furthermore it has been shown that the synthetic rate per cell in vitro may vary 20-fold between dif ferent clones (17) It is thus not surprising that the correlation between calculated synthetic rate of the M-component and the number of IgCC was weak at least when only the myeloma cases were con sidered The IgCC had the morphologic appear ance of plasma cells or plasmacytoid lymphocytes It should be noted that their number was always somewhat in excess of the number of plasma cells calculated in Pappenheim-stained bone marrow smears indicating that all the myeloma cells appear to synthesize Ig

The serum level of Ig classes other than that of the M-component was considerably depressed in the myeloma group and to a lesser extent in the BMG group The number or IgCC positive for these Ig classes was correspondingly diminished and there was a significant positive correlation between the calculated synthetic rate and the number of IgCC of these Ig classes in the bone marrow The total bone marrow cellularity was not known and the absolute number of Ig-containing bone marrow cells could thus not be determined but there is no indication that the bone marrow cellularity exclud ing the M-component producing clone is increased in monoclonal gammapathy It therefore seems *that an important factor causing a depressed serum* level of Ig classes other than that of the M-compo nent is a reduced synthetic rate secondary to a reduced number of cells belonging to other clones

The mean frequency of IgCC positive for the Ig class of the M-component was similar in IgA IgM and IgG monoclonal gammapathy (Table VI) On the other hand the calculated daily synthetic rate varied greatly being highest in IgA lowest in IgM and intermediate in IgG monoclonal gammapathy These discrepancies might be partly explained by differences in plasma volume and tissue distribution of IgCC in the three types of monoclonal gamma pathy It is well known that the plasma volume is often considerably increased in IgM monoclonal gammapathy It is less often increased in IgA and IgG monoclonal gammapathy and these Ig classes *do not differ in this respect (1)* The daily synthetic rate per cell may also be lower in IgG and IgM

than in IgA monoclonal gammapathy Such a difference between IgA and IgG myeloma has been observed by others (5) It should be noted that for Ig classes other than that of the M component differences in the mean number of IgCC were roughly parallelled by differences in daily synthetic rate (Table VII) There are also indications that the synthetic rates of IgA IgM and IgG per cell are similar in patients without monoclonal gammapathy (7 20) It might be that the cells in IgG and IgM monoclonal gammapathy tend to be less mature with respect to Ig synthesis and secretion than cells in IgA monoclonal gammapathy

The clinical usefulness of the immunofluorescent examination of bone marrow cells for the detection of intracellular Ig is illustrated by six cases included in the present study Although routine examinations of serum and urine failed to show any monoclonal immunoglobulin all had a monoclonal distribution profile of bone marrow IgCC On reinvestigation of serum and urine small amounts of monoclonal kappa chains could be demonstrated in three cases In another case trace amounts of monoclonal kappa chains could be detected in the urine at a later stage of the disease These patients all had clinical signs of multiple myeloma with widespread osteolytic lesions and increased number of immature plasma cells in the bone marrow All had bone marrow IgCC positive only for kappa chains These cases should be termed low secretory myeloma and will be the subject of a separate report (21)

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Clinical Features of Patients with a Serum M Component

Soila Peltonen,[1] Curt Wasastjerna[2] and Odd Wager[3]

From the City Hospitals and the Municipal Bacteriological Laboratory, Helsinki, Finland

ABSTRACT. The series comprised 169 patients with a serum M component (MC). Their mean age was 67.9 years. Eighty patients (47.3%) had a primary malignant lymphocytic or plasmacellular disease (68 myeloma, 6 macroglobulinaemia, 4 lymphoma, 2 chronic lymphocytic leukaemia). In 88 patients the MC was considered secondary and in one patient it was probably primary benign. In the group with a secondary MC the most common diagnoses were cardiovascular disease, an immunological disorder, infection, diabetes, and carcinoma. The possibility of a causal relation between cancer or prolonged antigenic stimulation and emergence of M components is discussed. Among 14 patients with definite myeloma the mean serum concentration of MC was 35.7 g/l, among 32 with secondary MC 12.9 g/l. Of the 68 patients with myeloma, 60% had an IgG MC, 22% an IgA MC and 18% an MC composed only of light chains. Of the patients in whom this paraprotein was secondary, it was IgG in 77%, IgA in 14% and IgM in 9%. One patient in this group had Bence Jones proteinuria, none had a pure light chain MC. Among patients with IgG or IgA myeloma or with a secondary MC and in whom the light chain type was identified, the kappa: lambda ratio was 1.5:1. Of the 11 patients with demonstrated light chain myeloma, the MC was of the kappa type in 4 and of the lambda type in 7. The levels of 'background' immunoglobulins were depressed in 88% of patients with a lymphocytic or plasmacellular neoplasia and in 38% with a secondary MC. Of the 91 patients whose serum samples were assayed for antibody activity, high titres were observed in 10.

M components (MCs), monoclonal immunoglobulins (Igs) that are discernible as spikes in free electrophoresis and as narrow bands in filter paper and cellulose acetate electrophoresis, were first identified in the serum of patients with multiple myeloma and Waldenström's macroglobulinaemia (4, 25). Both disorders represent a neoplastic proliferation of mutant clones of immunocytes. MCs were subsequently detected in other immunocytomas, such as heavy chain disease, chronic lymphocytic leukaemia and lymphoma (1, 18, 30), in a wide variety of other diseases (2, 5, 6, 9, 14, 19, 24, 28, 30) and even in apparently healthy persons, particularly the elderly. In the widely known population study by Axelsson et al. (3) the overall incidence of MCs was 0.9% and in the age group 80–90 years 5.7%.

An MC detected electrophoretically is usually typed by immunoelectrophoresis. But conventional electrophoresis may not always be sufficiently sensitive to detect the presence of MCs. Even when results are negative with this technique, small quantities of MC are sometimes revealed as deviations of the Ig precipitin lines in immunoelectrophoresis (11). According to Zawadski and Edwards (30), the rate at which MCs are detected can be doubled if screening includes both electrophoresis and immunoelectrophoresis.

Michaux and Heremans (14) classified the monoclonal immunoglobulin disorders as primary malignant, secondary, and primary benign. Hobbs (9) distinguished between malignant and benign immunocytomas. The diseases mentioned here in the opening paragraph are all considered primary malignant. However, the malignancy or benignity of certain disorders is not always self-evident, as in the case of primary amyloidosis and lichen myxedematosus. Zawadski and Edwards (30) classified these disorders as malignant. In spite of this ambiguity and even though the secondary nature of an

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Present addresses

[1] Salo District Hospital, SF-24130 Salo 13, Finland

[2] Vasa Central Hospital, SF-65130 Vasa 13, Finland

[3] Aurora Hospital, SF-00250 Helsinki 25, Finland

Table I *Main categories of disease associated with a serum M component represented among 169 patients*

	No	%	Total No	Total %
Primary malignant (lymphocytic plasmacytic neoplasias)			80	47 3
Multiple myeloma	68	40 2		
Macroglobulinaemia	6	3 6		
Lymphoma	4	2 3		
Chronic lymphocytic leukaemia	2	1 2		
Secondary			88	52 1
Amyloidosis	2	1.2		
Cancer	13	7 7		
Chronic infections	14	8 2		
Acute infections	5	3 0		
Immunological diseases	18	10 6		
Miscellaneous	36	21 3		
Primary benign (essential benign monoclonal gammapathy)			1	0 6

MC in association with a certain disease cannot usually be demonstrated beyond doubt we have d here the classification suggested by Michaux d Heremans MCs in malignant diseases with proliferation of cells not known to produce Ig are classified as secondary

The purpose of this study was to determine—for a group of patients with a serum MC—their clinical condition their age distribution the serum concentration and immunological type of the MC the levels of background Igs and antibody activity

PATIENTS AND METHODS

Of all the serum samples sent between Aug 1964 and Jan 1976 from four Helsinki City Hospitals to the Municipal Bacteriological Laboratory Helsinki for immunoelectrophoretic analysis 169 samples that contained an MC were from persons on whom enough clinical information was available to permit an assessment of their clinical features All patients were adults The mean follow up time was 20 1 months (range 1–90) Post mortem reports were available for 64 of the 169 cases

The 169 serum samples and 29 samples of urine (concentrated 25–50-fold) were analysed by immunoelectrophoresis as well as by cellulose acetate membrane electrophoresis (Microzone®) The serum concentrations of IgG IgA and IgM were estimated semiquantitatively by comparing the immunoelectrophoretic patterns of the test sera with those of pooled normal human sera on the same slide. In some instances the Ig levels were also assayed by radial immunodiffusion (12) In 91 sera antibody activity was assayed as described elsewhere (24)

The diagnoses entered in the hospital records were evaluated critically by the two clinicians (C W and S P) The minimum information compiled for each patient was. ESR blood count serum creatinine value and the results of the heat precipitation test for Bence Jones protein of a bone marrow aspiration biopsy and of a skeletal X ray examination

Of the 169 patients 68 were diagnosed as having myeloma a diagnosis that was accepted if the criteria of Wintrobe et al (29) were fulfilled Of these 68 cases the diagnosis was confirmed at autopsy in 27 This group of 68 patients included eight in whom myeloma was strongly suspected but not confirmed

Macroglobulinaemia was diagnosed if the serum contained a large IgM type MC fraction and if the bone marrow was infiltrated by lymphocytes of characteristic appearance while no lymphocytosis was present in the blood Of the six patients with macroglobulinaemia this diagnosis was confirmed by serum ultracentrifugation in one and at autopsy in two

RESULTS

Sex and age distribution

In this series the sexes were almost equally represented 56 8% were women and 43 2% men Among the patients with myeloma 64 7% were women The mean age for the whole series and for the myeloma group was virtually the same 67 9 and 67 years respectively

Diagnoses

Most patients had more than one illness Those with myeloma had a mean of 1 5 other diagnoses on the records Among those with a secondary MC the average number of illnesses diagnosed per patient was 2 3 Table I shows the main disease categories represented in the whole series

The number of patients with a primary malignant MC and with a secondary MC was almost the same 80 and 88 respectively In the former group 85% had multiple myeloma and 7 5% macroglobulinaemia Patients with a secondary MC had a variety of diseases—all listed in Table II Table II also compares the incidence of various diseases in the two main patient groups Infections immunological diseases carcinoma and diabetes occurred more often in patients with a secondary MC whereas cholelithiasis and thyroid disturbances (not including thyreoiditis which is here considered an immunological disease) were somewhat more frequent among those with myeloma These differences were not statistically significant

Of the patients with a secondary MC 13 had cancer Table III correlates the type of their MC

Table II *All recorded diagnoses associated with multiple myeloma and with secondary M component disorders among 156 patients*

	Myeloma (68 pats)		Secondary M component (88 pats)	
	No	%	No	%
Amyloidosis	3	4 4	2	2 2
Cancer	5	7 3	13	14 8
Chronic infections	16	23 5	34	38 6
Respiratory	4		10	
Urinary	5		6	
Intestinal	1		1	
Gallbladder			1	
Pancreas			2	
Skin & superficial veins			6	
Heart (rheumatic fever)	3		2	
Syphilis			2	
Tuberculosis	3		4	
Acute infections	11	16 1	18	20 4
Respiratory	7		10	
Urinary			2	
Liver			5	
Pancreas	1			
Meningitis	1			
Skin & superficial veins	2		1	
Immunological diseases	6	8 2	21	23 8
Rheumatoid arthritis	3		13	
Other connective tissue diseases	1		4	
Thyroiditis	1		1	
Cryoglobulinaemia			1	
Pernicious anaemia			1	
Nephritis	1			
Idiopathic thrombocytopenia			1	
Cardiovascular disease	26	38 2	46	52 2
Diabetes	6	8 8	17	19 3
Cholelithiasis	12	17 6	12	13 6
Goitre (including hyper and hypothyroidism)	12	17 6	8	9 1
Liver cirrhosis			2	
Bronchial asthma	1		2	
Erythema nodosum			2	
Malabsorption			1	
Hyperlipidaemia			2	
Megaloblastic anaemia			2	
Iron deficiency anaemia			8	9 1
Myelofibrosis			1	
Gastric ulcer	2		5	5 6
Benign tumours	4		9	10 2
Nephropathia	1		1	

with the site of the neoplasm Of the patients with myeloma two had cancer at the time of this study and three had been treated successfully for cancer These three patients are described briefly below

Case 1 This woman had been in good health until she underwent a mastectomy for mammary cancer in 1945 at the age of 46 Five years later her other breast was removed for the same reason In 1970 1 month after myeloma of the IgG lambda type had been diagnosed it was confirmed at autopsy

Case 2 In 1966 at the age of 62 this woman underwent a nephrectomy for cancer of the kidney Two years later liver metastases were suspected and treated with radiotherapy In 1971 an IgG kappa type myeloma was diagnosed When she died 6 months later the cause of death was thought to be disseminated tuberculosis The diagnosis of myeloma was confirmed at autopsy but there were no signs of carcinoma

Case 3 In 1954 when 56 years old this woman had a melanoma removed from her back In 1972 she died of an *IgG kappa type myeloma diagnosed 5 months earlier*

Table III *Correlation of site of tumour with type of serum M component in 13 patients in whom evidence of lymphoproliferative disease was lacking*

Site of tumour	No of pats	Type of M component
Prostate	1	IgM κ
Bronchus	1	IgG λ
Breast	2	IgG κ IgG λ
Colon	2	IgG λ *IgG (light chain* not identified)
Stomach	1	IgG λ
Pancreas	2	IgG λ IgG κ
Kidney	2	IgG κ (both)
Liver	2	IgG κ (both)

In this series only one patient had a transient MC

Case 4 A man born in 1896 had suffered since his 30s from recurrent respiratory infections In 1941 he was operated on for a gastric ulcer In Dec 1969 during an examination for low back pain serum electrophoresis and immunoelectrophoresis revealed an MC of the IgA lambda type Total serum IgA reached a concentration of 30 g/l serum concentrations of IgG and IgM were normal The number of plasma cells in the bone marrow was notably increased and showed a variable morphology In March 1970 the MC was no longer detectable by either cellulose acetate electrophoresis or immunoelectrophoresis The serum IgA level had dropped to 5 4 g/l and although still more than normal the plasma cells in bone marrow had a more normal appearance

Only one person had no apparent past or present illness

Case 5 This healthy 23-year-old man had given blood for laboratory control purposes when his IgG lambda type MC was accidentally detected The serum concentration at that time was 4 7 g/l Almost 2 years later it was 8 2 g/l and his bone marrow contained about 3% normal plasma cells All other laboratory values have remained normal and this man s general condition is still excellent

Table IV *Levels of background Ig in 169 patients with a serum M component*

	No of pats	Background Ig levels		
		Normal	Depressed	Not measured
Primary malignant				
Myeloma	68	5	53	10
Macroglobulinaemia	6	2	4	
Lymphoma	4	2	1	1
Chronic lymphocytic leukaemia	2		9	
Total	80	9	60	11
Secondary				
Amyloidosis	2	1	1	
Cancer	13	9	3	1
Infections	19	7	10	2
Immunological diseases	18	10	5	3
Miscellaneous	36	22	11	3
Total	88	49	30	9
Primary benign	1	1		

mmunoglobulins

For 50 patients from one hospital the quantity of the MC was calculated from the cellulose acetate electrophoresis curve For the 14 patients in this group who had myeloma the mean concentration (±S D) was 35 7±11 8 g/l for the 4 patients with probable myeloma it was 15 2 g/l (number of patients too small to calculate S D), and for the 32 patients with benign or secondary MC 12 9±5 9 g/l The difference in concentration between the first and third group is highly significant ($p<0\ 001$)

The levels of background Ig i e the levels of IgG, IgA and IgM not involved in the MC were depressed in 87% of patients with primary malignant MC and in 38% of those with secondary MC (Table IV) Table V correlates with the main associated disease categories the Ig class and the light chain type when identified of all MCs detected Of the 68 patients with myeloma 60% had an IgG MC 22% an IgA MC and 18% an MC composed only of light chains Of the patients whose MC was secondary it was IgG in 77% IgA in 14% and IgM in 9% No patient in this group had a micromolecular (pure light chain) MC For 137 patients the light chain of the MC was identified (Table V) for 32 patients it was not identified with certainty or not looked for In myeloma with an IgG or IgA MC the kappa lambda ratio of the 43 light chains identified was 1 5 1 Of the 11 myeloma patients who had a micromolecular MC and in whom the light chain was identified 4 had a kappa and 7 a lambda light chain Hence the kappa lambda ratio among myeloma patients was 1 3 1 For the 77 patients with secondary MC for whom the light chain type was identified this ratio was 1 5 1

The classical heat precipitation test for Bence

Table V *Correlation of main disease categories with the Ig class and light chain type of the serum M components present in 169 patients*

NI=not identified

	IgG			IgA			IgM			Micromolecular*		
	κ	λ	NI	κ	λ	NI	κ	λ	NI	κ	λ	NI
Myeloma	17	14	10	9	3	3				4	7	1
Macroglobulinaemia							1		5			
Lymphoma		2					1	1				
Chronic lymphocytic leukaemia			1									1
All primary malignant	17	16	11	9	3	3	2	1	5	4	7	2
Amyloidosis		2										
Cancer	6	5	1				1					
Infections	6	6	2		2		3					
Immunological disease	7	5	3	1	1		1					
Miscellaneous	13	9	3	4	4		1		2			
All secondary	32	27	9	5	7		6		2			
Primary benign		1										

* Light chains only

Table VI Patients with a serum M component and raised antibody titres

Pat no	Diagnosis	M component	Antibody	Titre
I	Myeloma	IgA κ	Anti glomerular	1 10 000
			Anti smooth muscle	1 100 000
II	Myeloma	IgA λ	Anti streptolysin	1 2,0 000
III	Myeloma	IgG λ	Anti thyroglobulin	1 2 500 000
IV	Lymphoma	IgM λ	Waaler Rose	1 1 000
V	Renal cancer	IgG κ	Anti nuclear IgG	1 1 280
			Anti nuclear IgM	1 320
VI	Stomach cancer	IgG λ	Anti smooth muscle	1 1 000
			Anti glomerular	1 100
VII	Pernicious anaemia	IgG λ	Anti parietal cell	1 1 000
VIII	Collagenosis"	IgG λ	Anti nuclear	1 10 000
IX	Rheumatoid arthritis	IgM κ	Anti parietal cell	1 1 000
X	Proteinuria	IgG λ	Wassermann reaction	1 1 000
	Nodular goitre		Anti thyroglobulin	1 800

Jones protein was positive for 22% of those with myeloma The urine of only 29 patients was studied immunoelectrophoretically Light chains were detected by this method in another 7% of the myeloma patients and hence at least 29% of the patients with myeloma had Bence Jones proteinuria

This paraprotein was detected in only one non myeloma patient

Case 6 In 1953 this woman born in 1909 had a cholecystectomy and in 1968 diabetes was diagnosed In May 1972 she was examined for marked weight loss and a deteriorated general condition ESR was 62 mm/h Hb 138 g/l serum creatinine 203 μmol/l (normal upper limit 120) and Bence Jones protein was detected by the heat precipitation test Serum electrophoresis and immunoelectrophoresis revealed an MC of the IgG lambda type Serum levels of IgA and IgM were depressed The number of plasma cells in bone marrow was slightly raised In Oct 1972 the patient died an autopsy revealed amyloidosis in various organs but nothing indicating myeloma

Of the 91 patients whose serum samples were assayed for antibody activity high titres were observed *in 10 (Table VI) Three of these 10 patients* had myeloma and have been described in detail elsewhere (22)

DISCUSSION

Most serum MCs detected in population studies are benign (3 7 23 24) In reported analyses of serum samples from hospitalized patients the proportion of primary malignant cases has ranged from 50 to 74% (2 5 9 10) In our series 47% of patients with a serum MC had a lymphocytic or plasmacellular neoplasia with multiple myeloma being the most common diagnosis It must be emphasized however that our series does not represent a random sampling of hostpitalized patients the serum samples of most patients were analysed because myeloma or a related disease had been suspected

Because healthy persons are not included in a hospital series the incidence in such a series of true primary benign MCs i e MCs occurring in healthy persons is probably zero One such case was however detected accidentally in our series when an MC was identified in the serum of one of a group of healthy persons from whom blood had been drawn to obtain normal control serum values

All the other MCs detected in our series are defined as secondary the most common accompanying diagnoses being cardiovascular disease chronic or acute infections diabetes immunological disease and carcinoma All these are common diseases particularly among the elderly in whom the presence of serum MCs is not rare (3 8) Although the difference in the frequency of these diseases between patients with secondary MC and those with myeloma is not statistically significant the higher frequency in the former group favours the concept that secondary MCs stem from the primary disease In a patient with an illness as serious as myeloma however other diseases might be inadvertently overlooked Hence we do not know how many MCs in our series were in fact secc

ary i e caused by the accompanying disease To determine the true incidence of secondary MC in a given disease for example diabetes a large series of diabetics would have to be compared for this paraprotein with an age matched group of healthy persons Few such studies have been done

Particularly interesting was the incidence of MCs associated with malignant neoplasms other than immunocytomas because it implicates the immune mechanisms operative in cancer In their review of the literature before 1972 Zawadski and Edwards (30) found the incidence of cancer in published series of monoclonal immunoglobulinaemia to range from 11 to 26% Their own experience showed this incidence to be 30% or higher

Non lymphoproliferative malignant tumours were found in 36 (16 1%) of the 224 patients with a serum MC studied by Colls and Lorier (5) but in only 72 (5 9%) of the 1242 patients with this paraprotein studied by Ameis et al (2) Our series included 18 patients (10 6%) with cancer five of whom also had myeloma If patients with myeloma nd other lymphoproliferative disorders are ex ded from our series then out of 89 patients h an MC 13 (14 6%) had cancer Screening serum samples of 5066 persons with a known or suspected neoplasm for MC by agar gel electrophoresis Migliore and Alexanian (15) found only 33 patients (0 06%) with a non myelomatous ma lignant neoplasm who had an MC These authors compared their results with the incidence of MCs and the age distribution in the population study by Axelsson et al (3) and concluded that the associa tion between MC and cancer was fortuitous rather than etiological The true incidence of MCs in can cer might however be higher than the aforemen tioned report suggests if the screening procedure were to include immunoelectrophoresis (30) Of the 67 patients with a serum MC studied by Williams et al (28) 14 had carcinoma Immunofluorescent examination of tumourous tissue from five of these 14 patients revealed infiltration of plasma cells filled with monoclonal Ig This finding is so remarkable that in spite of the conflicting results of Migliore and Alexanian (15) the possibility of a cause-effect relationship between cancer and MC must remain an open question—whose resolution awaits the results of further studies

Because the concentration of MC is usually higher in myeloma than in primary benign and secondary cases (23) the quantity of MC is considered to be of diagnostic importance In our series too although some overlapping occurred patients with myeloma had the highest serum concentrations of MC Appreciable amounts of free light chains are more often present in primary malignant cases and hence such chains are also diagnostically important (2) Free light chains were detected in 29% of our patients with multiple myeloma but urine immunoelectrophoresis was not performed in all

In one patient with primary amyloidosis the Bence Jones heat precipitation test was positive urine immunoelectrophoresis was not performed The distribution of Ig classes among patients with primary malignant and secondary MC was similar The kappa lambda ratio was almost the same in both groups about 3 2 and hence slightly lower than that usually reported (2 11 18) In the 11 cases with a pure light chain MC all of them malignant the ratio was reversed This finding is perhaps of limited value because of the smallness of the sample It does however agree with the observation of Stone and Frenkel (21) whose series of 35 patients with light chain myeloma included 16 patients with a kappa and 19 with a lambda light chain MC Ritzmann et al (19) reported a reversal of the usual kappa lambda ratio in a small group of patients with idiopathic (asymptomatic) MC In our patients with a secondary MC however this ratio was 1 5 1

The levels of background Igs are thought to be diagnostically important because they are more often depressed in malignant monoclonal Ig disorders (14) than in cases of benign MC Waldenström (26) has pointed out however that decreased levels are often present even in benign cases In our series background Ig levels were depressed in 87% of patients with lymphocytic or plasmacellular neoplasias but in 38% of those with a secondary MC as well Hence although normal levels are uncommon in cases of primary malignancy the level of background Igs cannot be taken as an unequivocal diagnostic criterion Numerous authors have reported on the activity of MCs against naturally occurring antigens (13 16 17 22 27)—a finding of great theoretical interest and an indication perhaps that some MCs are causally related to repeated antigenic stimulation In our series too it is possible that some of the high antibody titres detected were due to antibody active MCs

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Surgical Treatment of Myelomatosis—A Review of 18 Cases

L Unander Scharin J G Waldenstrom and Olle Zettervall

From the Departments of Orthopaedic Surgery and Internal Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT The authors review 18 patients with multiple myeloma who had bone destruction of a kind that indicated surgical therapy Eight patients had paraplegic myelopathy and one had compression of the cauda equina Four of them displayed partial to complete regression One patient lived for 77 months after the operation, most of the time in excellent condition The operative technique is discussed, with laminectomy, exeresis, filling of bone with cement and, in some instances, mechanical support from metal plates Early diagnosis and operation is imperative, postoperative irradiation obligatory in severe cases Radiation alone may be the method of choice in early stages The other 9 patients were operated upon for bone destruction in the limbs A Moore operation on the destroyed hip was performed in one patient, who lived in excellent condition for about four years Active surgical therapy combined with radiation and cytostatics seems to be of value in many patients with multiple myeloma

Chemotherapy in myelomatosis has resulted in longer life expectancy and a marked improvement in the quality of life for the patient The treatment is however only palliative and even if pain and myeloma protein concentration recede established skeletal lesions generally persist and new bone destructions often appear during the course of therapy The prolonged duration of the disease may therefore expose the patient to an increased risk of surgical complications i e manifest or impending fractures of bone in limbs and in vertebrae with compression of peripheral nerves or medulla Such manifestations of myelomatosis are rarely presenting symptoms as generalized bone pain usually antedates other complications with months or years

The term surgical complication is perhaps somewhat controversial Definite indications for surgical treatment in these clinical situations cannot be established from published experience of surgery in myelomatosis Radiotherapy is at least theoretically appealing as myeloma is a radiosensitive tumour

In our experience medullary compression with signs of myelopathy such as paraparesthesiae paraparesis and sphincter disturbance tends to appear rather abruptly and progress rapidly Swift relief of the pressure on the medulla is all important for the successful outcome in acute medullary compression We have therefore preferred operative decompression even though radiotherapy without surgery may be gratifying in some cases (4)

As chemotherapy after restitution of paraparesis may give long survival operative treatment is ethically defensible if the patient s myelomatosis is only slightly or moderately advanced The immediate cause of medullary compression in myelomatosis is often an extradural plasmocytoma rather than collapse of a vertebra Experience of surgical therapy in other extradural malignant tumours seems to be favourable (3) and is a further stimulus to an operative approach in myelopathy of myelomatosis The same applies to supplementary postoperative radiotherapy

PATIENTS

We have reviewed all cases in Malmö suffering from myelomatosis with paraplegia symptoms or severe affection of the extremity skeleton during a ten year period (1965–75) Although there are too few patients to support far reaching conclusions about indications for surgical and radiological therapy in such cases we believe it is of interest to discuss our experiences Myelomatosis is a rare disease and comparatively little has been published about the handling of these types of complications Our patients comprise a consecutive unselected and therefore representative series

The patients who have been treated surgically in Malmö for spinal myeloma with neurological complications during 1965–75 are listed in Table I One other patient presented with paraparesis during this period but as she was in bad general condition due to advanced dise-

Table I. *Spinal myeloma: operative treatment*

Pat. sex year of birth	Approximate duration of neurological symptoms (d.)	Year of operation	Dominating symptoms	Localization	Operation laminectomy and	Postoperative regression of symptoms
S. J. ♀ 1900	21	1970	Cervical rhizopathy paraplegia	C VI	Exeresis stabilization	Partial initially then progression
Å. H. ♂ 1916	11	1968	Bladder paresis paraplegia	T I	Exeresis stabilization	Complete
B. L. ♂ 1919	5	1969	Paraplegia	T VIII T X	Exeresis bone cement stabilization	Partial walk with crutches
N. H. ♂ 1898	10	1970	Paraplegia	T II–IV	Exeresis	Partial
N. J. ♂ 1925	3	1974	Paraplegia	T IX	Exeresis	None
K. M. ♂ 1911	1	1974	Paraplegia	T II–III	Exeresis bone cement stabilization	None
S. N. ♀ 1908	11	1972	Paraplegia	T VIII	Exeresis stabilization	None
♂	14	1968	Paraplegia	T VI	Exeresis stabilization	Partial initially but paraparesis 1 week postoperatively
K. Ö. ♂ 1912	20	1974	Cauda equina compression	L II	Exeresis bone cement stabilization	Complete

eration was not performed. She died less than a month after the onset of medullary compression symptoms. Table II gives information on the patients who were operated upon for impending or completed fractures of extremity bones during the ten year period. Data on preoperative duration of disease (after diagnosis of myelomatosis), postoperative survival and immunochemical characteristics of M-components in plasma and Bence Jones protein in urine are given in Table III.

The diagnosis of myelomatosis has been established when at least two of the following criteria have been satisfied: a progressively increasing serum M-component, Bence Jones proteinuria together with hypogammaglobulinaemia, plasma cells in bone marrow aspirate >10% of nucleated cells and typical skeletal lesions on the bone X-ray.

Patients with previously known myelomatosis had all received melphalan or cyclophosphamide therapy in the preoperative period and all patients had postoperative cytostatics (7). At the time of operation one of the patients (K. M.) was uraemic and hypercalcaemic. The operative results are also presented in Tables I and II.

Surgical Aspects

Spine

At operation the compressing plasma cell tumour is usually seen as an outgrowth from the pedicle or the body of the affected vertebra and laminectomy is then the logical procedure in order to remove tumour tissue as extensively as possible. The vertebral body often appears totally destroyed and excavated by the tumour. In this situation the vertebral body or bodies might be filled with bone cement and the spinal processes joined by metallic osteosynthesis material or bone cement (Fig. 1). The medulla should be protected with autologous fatty tissue grafts. This is an important point as heat is generated during polymerization of the bone cement, but if protected as mentioned the medulla and spinal nerves seem not to be injured. In the thoracic region, stabilization from the ribs may be sufficient in some instances to render cementing of the vertebral body unnecessary. The operative procedure is laminectomy and stabilization of the spinal processes. In the cervical and lumbar regions the vertebral bodies should be filled with bone cement. As bleeding is often profuse and cementing an efficient haemostatic measure

Other treatment	Postoperative survival (mo)	Comments
Melphalan radiotherapy	9	Preoperative treatment with scull traction and radiotherapy
Melphalan radiotherapy	77	Fig 1 see also Table II
Melphalan	11	
Radiotherapy *melphalan*	9	
Melphalan	2	Local tumour *recurrence* reoperation after 4 weeks
Melphalan	2	Hypercalcaemia and uraemia at operation See also Table II
Melphalan radiotherapy	10	
Melphalan	1	Died uraemic and hyper calcaemic
Melphalan radiotherapy	24+	Reoperation fixation of osteosynthesis material

filling of thoracic vertebrae is sometimes also to be recommended Fixation of the bone cement in the vertebral body is of paramount importance and a special drill has been constructed to facilitate boring in the adjacent vertebral bodies

A myelomatous medullary compression may develop without any demonstrable lesion of the vertebra on plain X ray films or several vertebrae may be affected We have therefore considered emergency gas or contrast (metrizamide Amipaque®) myelography a necessary part of the preoperative diagnostic work-up for defining the affected spinal level It should be stressed that although clinical level diagnosis as judged from a sensory demarcation line may be possible in some instances poor correlation between dermatome and actually affected medullary segment is not infrequent in cord compression

Limbs

When tumour growth in an *extremity bone* has progressed to an impending or a completed fracture operative treatment aims at stabilization Conventional fixation techniques employing marrow nails and nails and plates are useful as is filling of tumour cavities with bone cement (Fig 2) If the lesion is localized in a proximal joint arthroplasty may be required (Fig 3)

In both "spinal" and "extremity" cases surgical treatment offers the advantage of early ambulation Neurological rehabilitation with walk exercises bandages and basin training can usually be instituted in the first postoperative day(s) and walking could be encouraged almost immediately in most cases of lower extremity affection As a rule we have been able to continue cytostatic treatment during postoperative radiotherapy—contrary to expectation—*since marrow toxicity has not been especially* prominent in combined modality treatment

DISCUSSION

Spinal myeloma

In the city of Malmö (250 000 inhabitants) about 10 new myeloma cases were found annually The diagnosis of myeloma was unknown in only one person with paraparesis the real cause being found at operation This means that paraparesis was the *initial symptom of myeloma in only 1% of our pa*tients from 1965–75 which is a much lower figure than that (6%) found in the Mayo Clinic The latter patients from the years 1930–50 were diagnosed according to other criteria Only one of our eight patients with cord compression had already been known to suffer from myeloma before the ten year period we are investigating It may well be that the risk of developing myelopathy should be calculated at about 5% There was no obvious connection between the length of time that the diagnosis of myeloma had been established preoperatively and the postoperative survival which varied between 1 and 77 months The total duration of disease from the first diagnosis to exitus varied between 7 and 193 months (mean 68 5 median 62 5) thus surpassing the figures for the total myeloma population in Malmö treated with cytostatics This fact speaks against the assumption that myelopathy occurs in patients with an especially malignant type of the disease It seems to us more probable that long survival in a relatively benign myeloma should carry an increased risk of tumour pressure on the spinal cord at some time during the disease In the group spinal myeloma there is one patient (K Ö) with compression of the cauda equina The preoperative duration of his neurological symptoms was rather long 20 days

The preoperative duration of the myelopathy is not quite clear in patient S J as she was relatively immobile owing to generalized pain and symptoms–

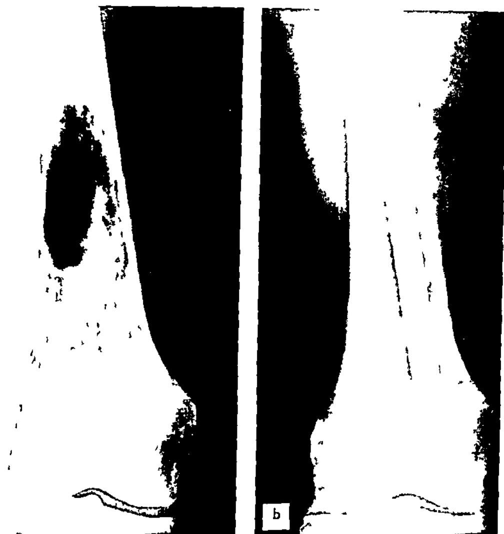


Fig 2 Myeloma in the distal part of right femur diaphysis (*a*) After stabilizing with Kuntscher nail (*b*)

ment of choice in cases of medullary compression with minor neurological symptoms of slow progression and in more serious cases where operation is contraindicated Swift relief of medullary compression is all important however for recovery of motor function when this is seriously impaired Therefore topical diagnosis of the lesion and operation must still be considered as emergency measures when motor symptoms show rapid progression as in our patients

Judging from our results the case for an immediate operative approach is admittedly not too strong In principle better results should be obtained if the interval between the start of medullary compression symptoms and operation could be shortened We therefore propose that patients with myelomatosis should be instructed to report localized back pain with radicular extension paresthesiae or weakness in extremities and sphincter symptoms *immediately* At control examinations patients should be questioned about such symptoms and a neurological examination should be performed routinely Other therapeutic approaches may be effective in special circumstances for instance after known trauma as illustrated by the following history

M G a woman aged 56 with myelomatosis affecting the cervical spine known for one year

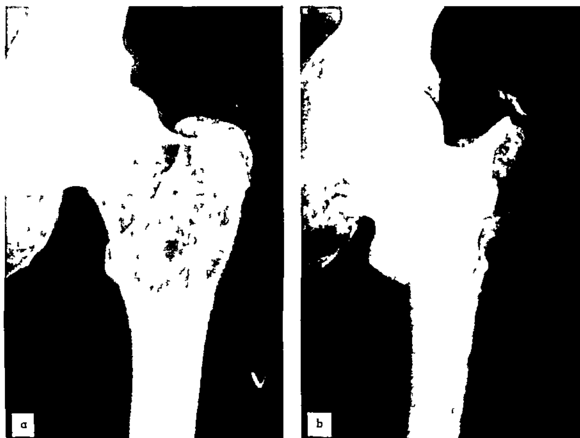


Fig 3 Myeloma in the upper part of left femur (*a*) After resection of the tumour (*b*) Arthroplasty according to Moore with bone cement

Cervical pain and quadriplegia appeared immediately after a forced dorsal flexion of the head Traction and radiotherapy resulted in complete restitution of the neurological symptoms The patient lived one year after the traction

Limbs

With or without postoperative radiotherapy in 8 of the cases good results were obtained with osteosynthesis or resection of tumorous bone and insertion of a prosthesis A fair result was noted in the patient operated upon according to Girdlestone Prophylactic operation was performed when the extent of the lesion was such that a fracture seemed impending irrespective of whether local pain was troubling or not In at least some of these patients a fracture has probably been avoided We feel that prophylactic operation should be performed when extremity bone fracture seems imminent in view of the long postoperative survival in many patients We believe that an X ray is needed as soon as the patient complains of localized persistent pain in an extremity in order to detect an operable bone destruction

Operative fixation of established fractures is also gratifying and aids in rapid mobilization of the patient This is an important point as a bedridden myelomatosis patient is in danger of hypercalcaemia The patients—both those with spinal and those with extremity bone affection—have received intensive physiotherapy A high fluid intake has been encouraged and with this regime postoperative hypercalcaemia has not developed

Table III *Preoperative duration of myelomatosis, postoperative survival time and type of M component and Bence Jones protein*

K=kappa L=lambda n d =not done

Pat	Preoperative duration[a] of myelomatosis (mo)	Postoperative survival[b] (mo)	Type of serum M-component conc (g/l)	Bence Jones protein type	Case appearing in Table
S J	42	9	GK 14	+K	I
Å H	0	77	AK 32	+K	I II
B L	43	11	GK 16	(+)	I
N H	0 3	9	–	+L	I
N J	5	2	AL 54	–	I
K M	141	52	–	+K	I II
S N	4	10	–	+L	I
K Ö	0 5	24+	GK 22	+K	I
B N	17	1	GK 41	+K	I
T N	9	3	AL 44	+L	II
A M H	*12*	*30*	*GL 52*	*+n d*	*II*
Å N	6	7	AK 73	–	II
J W	1	24	GK 18	–	II
L R	4	5	AK 25	–	II
G S	0 3	25+	GK 63	+K	II
T S	*9 5*	*60+*	*GK 70*	–	*II*

[a] After diagnosis of myelomatosis to first operation in Table I or II
[b] After first operation (in Table I or II)

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Extreme Hyponatremia in Patients with Myelomatosis

An Effect of Cationic Paraproteins

B Bloth T Christensson and H Mellstedt

From the Department of Medicine Serafimerlasarettet Stockholm Sweden

ABSTRACT Three patients with IgG myelomatosis and extreme hyponatremia are described By isoelectric focusing of the M component it is demonstrated that the subnormal sodium value is most likely explained by a cationic effect of the myeloma globulin

Myelomatosis associated with hyponatremia has been reported earlier (8 9) But the phenomenon has been little emphasized and may be more common than indicated so far It is important to realize that patients with this type of hyponatremia are asymptomatic and should not be treated with sodium replacement which may give rise to over loading

The present report describes three cases with myelomatosis and extremely low sodium values without neurological or psychiatric symptoms

METHODS

Serum immunoglobulins were determined by single radial gel diffusion technique (4) (normal ranges (g/l) IgG 9-18 IgA 1 5-3 0 IgM 0 5-1 5)

The IgG M components from cases 1 and 3 were purified by chromatography on Sephadex A 25 (Pharmacia Uppsala Sweden) and then submitted to thin layer isoelectric focusing in polyacrylamide gel (10) Serum sodium was recorded by AutoAnalyzer (Technicon Instruments Tarrytown USA) (normal range 136-148 mmol/l)

CASE REPORTS

Case 1

A 73 year-old previously healthy man was admitted to the hospital due to the finding of an IgG M component on serum electrophoresis He was in good condition with no psychiatric or neurologic symptoms but complained of fatigue and shortness of breath BP 130/80 mmHg Laboratory tests ESR 131 mm/h Hb 105 g/l WBC 6.3×10^9/l with a normal differential count platelet count 100×10^9/l Bone marrow biopsy revealed a highly differentiated plasma cell myeloma The plasma cells constituted about 60% of all nucleated cells X ray examination showed typical myeloma changes of the skull Quantitative Ig analyses IgG 50 g/l and subnormal levels of IgA and IgM

Initially serum sodium and potassium values were 117 and 3 5 mmol/l respectively Repeated sodium analyses on the following days showed values around 110 mmol/l Serum calcium chloride alkaline phosphatases lipids and blood sugar were normal Diurnal serum cortisol values were normal as was urinary excretion of 17 keto and 17 ketogenic steroids Routine kidney and thyroid function tests were without remarks As soon as the diagnosis had been confirmed melphalan (0 25 mg/kg/day) and prednisolone (2 mg/kg/day) were instituted for four days and repeated every sixth week After a few courses the patient improved and an increase in serum sodium values was noted However the levels were still subnormal though never below 125 mmol/l

Two and a half years later the disease progressed and the patient died due to bleedings Post mortem examination showed myeloma cell infiltration of the bone marrow minimal myeloma changes in the kidney but no abnormalities in the brain or in the adrenals

Case 2

A previously healthy 65 year old woman was remitted to the clinic because of lumbar pains and anemia Laboratory investigations ESR 180 mm/h Hb 76 g/l WBC and platelet counts normal Differential count of peripheral leukocytes showed 7% plasma cells 80% myeloma cells were seen in a bone marrow specimen Serum electrophoresis revealed an M component of IgG class Quantitation of immunoglobulins IgG 100 g/l IgA and IgM subnormal values Serum creatinine 160 μmol/l Serum sodium potassium chloride alkaline phosphatases serum lipids and blood sugar were within normal limits Serum sodium 135 mmol/l and serum calcium 2 90 mmol/l Thyroid function was unaffected X ray examination of the skeleton showed no myeloma destructions Therapy with high dosage melphalan/prednisolone intermittently (see above) was started

Six weeks later a second cytostatic course was instituted Laboratory tests ESR 146 mm/h Hb 104 g/l serum calcium 2 70 mmol/l Serum chloride and potassium were normal but serum sodium had decreased to 120 mmol/l IgG in serum was 150 g/l (Fig 1)

After another six week period the patient was hos-

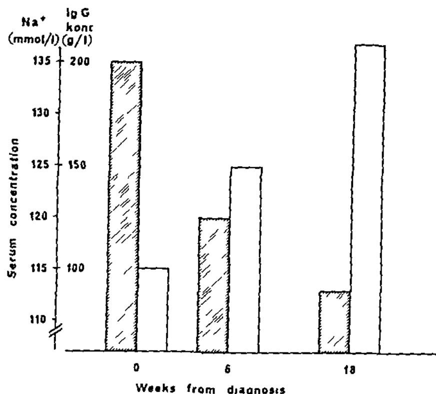


Fig 1 Variations in serum sodium (■) and IgG concentrations (□) during the course of the disease in case 2

for her third melphalan/prednisolone course this time the diurnal cortisol levels as well as 17 keto 17 ketogenic steroids were normal A salt losing nephropathy was excluded

Eighteen weeks from the time of diagnosis the patient was again hospitalized for cytostatic treatment Because of therapeutic failure she received cyclophosphamide instead of melphalan Laboratory investigations ESR 126 mm/h Hb 64 g/l WBC 3 6×10⁹/l serum creatinine 88 μmol/l serum sodium 113 mmol/l serum potassium 4 0 mmol/l IgG 210 g/l (Fig 1) During this hospitalization her condition deteriorated She developed fever and severe diarrhea and died shortly after Autopsy revealed normal adrenals and brain structures including the pituitary gland No abnormalities could be found on microscopical examination of the kidneys

Case 3

This patient was a 67 year-old woman admitted to the hospital because a typical myeloma picture had been found in the bone marrow and an IgG M-component in the serum. X ray examination showed characteristic myeloma changes in the skull During a period of 2½ years she was treated with melphalan/prednisolone and was kept in fairly good condition

Later on the disease progressed The patient developed multiple vertebral fractures Her IgG concentration in serum increased from 53 to 130 g/l Concomitantly serum sodium levels decreased and repeated analyses showed values around 110 mmol/l Laboratory tests ESR 150 mm/h Hb 70 g/l WBC about 3×10⁹/l platelet count 20×10⁹/l Routine kidney function tests were normal Diurnal cortisol curve and urinary excretion of 17 keto and 17 ketogenic steroids were normal No abnormal liver function could be detected Intermittently hypercalcemia was noticed (2 80–3 00 mmol/l) Later on the patient's condition deteriorated she developed generalized bleedings and died Post mortem examination revealed normal kidneys adrenals brain structures thyroid and para thyroid glands

RESULTS AND DISCUSSION

Hyponatremia has been described in connection with several diseases such as alcoholism intermittent porphyria hyperlipemia pneumonia CNS affections Addison's disease myxedema hypopituitarism tuberculosis various malignancies including oat cell carcinoma bronchogenic carcinoma and Hodgkin's disease as reviewed by Bartter and Schwartz (1) The pathophysiology of the hyponatremia in these cases is obscure It has been suggested that the underlying disease in some manner induces an inappropriate secretion of antidiuretic hormone (7) Acute myelocytic leucemia is sometimes associated with low serum sodium values In such patients it has been postulated that the hyponatremia may be caused by a natriuretic substance released by the leucemic cells (5)

Low serum sodium values are also found in pa

tients with hyperproteinemia and this has been explained by the volume-displacing effect of the proteins However Frick et al (3), in a study of hyponatremia in myelomatosis indicated that certain types of paraproteins behave as cations Thereby they exert a displacing effect on other cations especially sodium Murray et al (6) determined the isoelectric point of six patients with plasma cell myeloma and found distinct paraprotein bands at pH 7 5–9 0 clearly demonstrating them to be cationic at normal serum pH

In our study which has been performed retrospectively serum from two of the patients (cases 1 and 3) was obtainable and analyzed by isoelectrical focusing in polyacrylamide gel (10) In both cases the paraprotein peaks were found to have isoelectric points at pH above 8 5 and may therefore act as cation at normal pH and may displace sodium

Our three cases demonstrated no neurologic or psychiatric symptoms in spite of extreme hyponatremia Several serum sodium values around 110 mmol/l were recorded Interestingly it has been shown that subjects with such low concentrations usually are unconscious (3) Our three patients were in comparatively good condition apart from skeletal pains They were on a normal dietary sodium intake No histories of alcoholism could be revealed Clinical and laboratory investigations as well as autopsy ruled out pituitary thyroid and adrenal affections as the cause of hyponatremia A dilutional hyponatremia was excluded from clinical and laboratory findings The syndrome of renal sodium loss associated with inappropriate antidiuretic hormone secretion (7) is unlikely to be the cause since no pathological changes known to be connected with such a syndrome were found Nor could kidney function tests blood sugar levels or serum lipids be associated with current recordings of hyponatremia

It seems reasonable to assume that the extreme hyponatremic state of these three patients was associated with a cationic effect of the paraproteins It should be stressed that no sodium replacement therapy should be instituted in such cases

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Factors Responsible for Bone Marrow Toxicity after Treatment of Myeloma Patients with Different Alkylating Agents

U Ringborg and R Lewensohn

From Radiumhemmet Karolinska Hospital Stockholm Sweden

ABSTRACT Leukopenia and thrombocytopenia were studied after loading doses of melphalan (5 mg/daily for 18–25 days) in 71 myeloma patients Seventy per cent of the patients developed pronounced leukopenia (white cells $<2\ 0\times10^9/l$) and/or thrombocytopenia (platelets $<100\times10^9/l$) The patients with pronounced and moderate hematological side-effects, respectively, were compared for weight and age The body weight was the same in the two groups, indicating that the patient's weight is of minor importance for the dosage of melphalan There was a numerical difference in age, on the borderline for statistical significance, indicating that the age of patient may be of minor importance for dosage of melphalan It is possible that more nounced age differences may be of greater importance in this respect Fifteen patients with myeloma were treated with cyclophosphamide Compared with melphalan, the effect on white cells was the same, while the incidence of thrombocytopenia was statistically significantly lower with cyclophosphamide

Bone marrow toxicity limits the use of cytotoxic drugs in the treatment of malignant diseases Multiple myeloma is one of the diseases which respond well to alkylating agents and survival is prolonged by treatment with melphalan and cyclophosphamide (2 4) Patients vary greatly in their tendency to develop hematological side-effects after cytostatic treatment Melphalan causes leukopenia and thrombocytopenia In order to standardize the dosage this drug is given in relation to body weight or body surface But as with most other cytostatic drugs there is no rationale for this practice A number of myeloma patients who have received melphalan treatment have therefore been investigated regarding acute leukopenia and thrombocytopenia and the side effects have been related to the weight and age of the patients A comparison was also made between side effects of melphalan and cyclophosphamide

PATIENTS AND METHODS

In 71 patients with multiple myeloma treated by Professor Jan Waldenstrom at Malmo General Hospital the initial treatment consisted of a loading dose of melphalan 5 mg/day for 18–25 days After the loading dose the patients were followed regarding white peripheral cells and platelets and the nadir was registered Usually this occurred about one week after the end of loading When the blood values started to normalize, a continuous low dose of melphalan was administered

Fifteen multiple myeloma patients treated with cyclophosphamide either in daily oral doses or i v courses were compared with melphalan treated myeloma patients regarding hematological side-effects Some of these patients were treated in the Department of Medicine Malmö General Hospital some in Radiumhemmet Stockholm

RESULTS

The 71 melphalan treated patients were divided into two groups one—50 patients (70%)—with pronounced hematological side effects (white cells $<2\times10^9/l$ and/or platelets $<100\times10^9/l$) and the other—21 patients (30%)—with minor hematological side effects (leukocytes $\geq2\times10^9/l$ and platelets $\geq100\times10^9/l$) (Table I)

In Table II the patients with severe and moderate leukopenia and thrombocytopenia are compared regarding melphalan dose body weight and age Treatment had lasted 21 days for most patients but for various reasons its duration had been somewhat shorter or longer in a number of cases The total melphalan dose did not differ between patients with moderate and severe hematological side-effects This must mean that other factors than slight differences in total melphalan loading are responsible for the degree of hematological side-effects

Since the patients received 5 mg melphalan daily regardless of body weight it was possible to test

Table I *Peripheral leukocytes and platelets in myeloma patients with moderate and pronounced hematological side effects after loading dose with melphalan (mean ± S D ×10⁹ cells/l)*

	Before treatment	After treatment	% of pretreat ment value	*p*
Moderate side effects				
Peripheral leukocytes	5 01±1 01	2 85±1 03	60±19	<0 001
Platelets	340±106	187±35	66±22	<0 001
Pronounced side effects				
Peripheral leukocytes	5 07±1 19	1 28±0 47	27±14	<0 001
Platelets	200±81	47±46	23±19	<0 001

Table II *Comparison of melphalan dose weight and age in myeloma patients with moderate and severe leukopenia and thrombocytopenia after loading dose of melphalan*

	Moderate side effects	Severe side effects	Signifi cance of difference
Melphalan dose (mg)	111 9±15 8	107 2±16 5	n s
Weight (kg)	68 6±13 9	64 9±10 3	n s
Age (y)	61 6± 9 5	66 1± 9 7	n s

n s =Not significant ($p<0$ 1)

whether body weight influenced the side effects No difference in body weight was found between patients with moderate and severe leukopenia and thrombocytopenia (Table II) indicating that the body weight is not of major importance for the dosage of melphalan

It is a clinical observation that age is of im portance for side effects after cytostatic treatment The mean age of patients with severe hematological side-effects was 4 5 years higher than that of the patients with moderate side effects This difference is on the borderline for statistical significance ($p<0$ 1) indicating that age may influence the side effects but is of minor importance for the dosage of melphalan

Fifteen patients with multiple myeloma were treated with cyclophosphamide either in daily oral doses or i v courses All patients showed hematological side effects with leukopenia but the effect on the platelets was not significant (Table III)

In Fig 1 the hematological side effects after melphalan with a view to attaining a rapid tumor compared by measuring the correlation between leukocytes and platelets There is a statistically significant correlation between leukocytes and platelets after melphalan treatment (Fig 1*a*) ex pressed as per cent of the pretreatment value ($r=0$ 52 $p<0$ 001) No significant correlation was found when the same analysis was performed on patients treated with cyclophosphamide (Fig 1*b*)

DISCUSSION

Bone marrow toxicity limits the use of cytostatic drugs in the treatment of malignant diseases It is therefore of interest to determine which factors are of importance for this toxicity since clinical obser vations indicate that it varies greatly among pa tients After treatment with a loading dose of

Table III *Hematological side effects in myeloma patients after treatment with cyclophosphamide (mean ± S D ×10⁹ cells/l)*

	Before treatment	After treatment	% of pretreat ment value	Significance of difference
Peripheral leukocytes	4 238±1 96	1 446±0 760	39±21	$p<0$ 001
Platelets	157±54	120±53	78±23	n s

n s =Not significant

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Factors Responsible for Bone Marrow Toxicity after Treatment of Myeloma Patients with Different Alkylating Agents

U Ringborg and R Lewensohn

From Radiumhemmet Karolinska Hospital Stockholm Sweden

ABSTRACT Leukopenia and thrombocytopenia were studied after loading doses of melphalan (5 mg/daily for 18–25 days) in 71 myeloma patients. Seventy per cent of the patients developed pronounced leukopenia (white cells $<2.0\times10^9$/l) and/or thrombocytopenia (platelets $<100\times10^9$/l) The patients with pronounced and moderate hematological side-effects, respectively, were compared for weight and age The body weight was the same in the two groups, indicating that the patient s weight is of minor importance for the dosage of melphalan There was a numerical difference in age, on the borderline for statistical significance, indicating that the age of the myeloma patient may be of minor importance for the dosage of melphalan It is possible that more pronounced age differences may be of greater importance in this respect Fifteen patients with myeloma were treated with cyclophosphamide Compared with melphalan, the effect on white cells was the same, while the incidence of thrombocytopenia was statistically significantly lower with cyclophosphamide

Bone marrow toxicity limits the use of cytotoxic drugs in the treatment of malignant diseases Multiple myeloma is one of the diseases which respond well to alkylating agents and survival is prolonged by treatment with melphalan and cyclophosphamide (2 4) Patients vary greatly in their tendency to develop hematological side-effects after cytostatic treatment Melphalan causes leukopenia and thrombocytopenia In order to standardize the dosage this drug is given in relation to body weight or body surface But as with most other cytostatic drugs there is no rationale for this practice A number of myeloma patients who have received melphalan treatment have therefore been investigated regarding acute leukopenia and thrombocytopenia and the side-effects have been related to the weight and age of the patients A comparison was also made between side-effects of melphalan and cyclophosphamide

PATIENTS AND METHODS

In 71 patients with multiple myeloma treated by Professor Jan Waldenström at Malmö General Hospital the initial treatment consisted of a loading dose of melphalan 5 mg/day for 18–25 days After the loading dose the patients were followed regarding white peripheral cells and platelets and the nadir was registered Usually this occurred about one week after the end of loading When the blood values started to normalize a continuous low dose of melphalan was administered

Fifteen multiple myeloma patients treated with cyclophosphamide either in daily oral doses or i v courses were compared with melphalan-treated myeloma patients regarding hematological side-effects Some of these patients were treated in the Department of Medicine Malmö General Hospital some in Radiumhemmet Stockholm

RESULTS

The 71 melphalan treated patients were divided into two groups one—50 patients (70%)—with pronounced hematological side-effects (white cells $<2\times10^9$/l and/or platelets $<100\times10^9$/l) and the other—21 patients (30%)—with minor hematological side-effects (leukocytes $\geq2\times10^9$/l and platelets $\geq100\times10^9$/l) (Table I)

In Table II the patients with severe and moderate leukopenia and thrombocytopenia are compared regarding melphalan dose body weight and age Treatment had lasted 21 days for most patients but for various reasons its duration had been somewhat shorter or longer in a number of cases The total melphalan dose did not differ between patients with moderate and severe hematological side-effects This must mean that other factors than slight differences in total melphalan loading are responsible for the degree of hematological side-effects

Since the patients received 5 mg melphalan daily regardless of body weight it was possible to test

Table I *Peripheral leukocytes and platelets in myeloma patients with moderate and pronounced hematological side effects after loading dose with melphalan (mean ± S.D. ×10⁹ cells/l)*

	Before treatment	After treatment	% of pretreatment value	p
Moderate side effects				
Peripheral leukocytes	5.01 ± 1.01	2.85 ± 1.03	60 ± 19	[illegible]
Platelets	340 ± 106	187 ± 35	66 ± 22	[illegible]
Pronounced side effects				
Peripheral leukocytes	5.07 ± 1.19	1.28 ± 0.47	27 ± 14	[illegible]
Platelets	200 ± 81	47 ± 46	23 ± 19	[illegible]

Table II *Comparison of melphalan dose, weight and age in myeloma patients with moderate and severe leukopenia and thrombocytopenia after loading dose of melphalan*

	Moderate side effects	Severe side effects	Significance of difference
Melphalan dose (mg)	111.9 ± 15.8	107.2 ± 16.5	n.s.
Weight (kg)	68.6 ± 13.9	64.9 ± 10.3	n.s.
Age (y.)	61.6 ± 9.5	66.1 ± 9.7	n.s.

n.s. = Not significant ($p < 0.1$)

whether body weight influenced the side effects. No difference in body weight was found between patients with moderate and severe leukopenia and thrombocytopenia (Table II) indicating that the body weight is not of major importance for the dosage of melphalan.

It is a clinical observation that age is of importance for side effects after cytostatic treatment. The mean age of patients with severe hematological side effects was 4.5 years higher than that of the patients with moderate side effects. This difference is on the borderline for statistical significance ($p < 0.1$) indicating that age may influence the side effects but is of minor importance for the dosage of melphalan.

Fifteen patients with multiple myeloma were treated with cyclophosphamide either in daily oral doses or i.v. courses. All patients showed hematological side-effects with leukopenia but the effect on the platelets was not significant (Table III).

In Fig. 1 the hematological side-effects after melphalan, with a view to attaining a rapid tumor... compared by measuring the correlation between leukocytes and platelets. There is a statistically significant correlation between leukocytes and platelets after melphalan treatment (Fig. 1*a*) expressed as per cent of the pretreatment value ($r = 0.52$, $p < 0.001$). No significant correlation was found when the same analysis was performed on patients treated with cyclophosphamide (Fig. 1*b*).

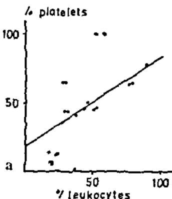
DISCUSSION

Bone marrow toxicity limits the use of cytostatic drugs in the treatment of malignant diseases. It is therefore of interest to determine which factors are of importance for this toxicity since clinical observations indicate that it varies greatly among patients. After treatment with a loading dose of

Table III *Hematological side effects in myeloma patients after treatment with cyclophosphamide (mean ± S.D. ×10⁹ cells/l)*

	Before treatment	After treatment	% of pretreatment value	Significance of difference
Peripheral leukocytes	4.238 ± 1.96	1.446 ± 0.760	39 ± 21	$p < 0.001$
Platelets	157 ± 54	120 ± 53	78 ± 23	*n.s.*

n.s. = Not significant



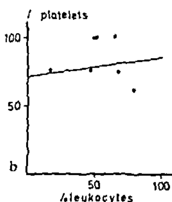


Fig 1 Correlation analysis of leukopenia and thrombocytopenia after treatment with melphalan (*a*) and cyclophosphamide (*b*) Each point represents the percentage of pretreatment value $r=0.52$ for melphalan ($p<0.001$) $r=0.13$ for cyclophosphamide (not significant)

melphalan with a view to attaining a rapid tumor response (4) 70% of the patients developed severe hematological side-effects defined as leukopenia with $<2\times10^9$ cells/l or thrombocytopenia with $<100\times10^9$ cells/l After melphalan treatment there is a good correlation between the decrease in white cells and platelets The absence of a difference in y weight between patients with severe and derate leukopenia and thrombocytopenia means t body weight is not of importance for the dosage of melphalan when administered as described here

The numerical difference in age between the two groups was statistically insignificant which must mean that age cannot be very important for the incidence of leukopenia or thrombocytopenia It is conceivable that age plays a greater part in diseases which affect people over a wider age range than the present myeloma patients but no conclusions about this can be deduced from our findings

The finding that melphalan and cyclophosphamide gave rise to the same degree of leukopenia and that cyclophosphamide caused significantly less thrombocytopenia agrees with an earlier report (3) We consider this observation important since other authors have been unable to find any such differences between these two drugs (1 3 4 5) If there is a difference one should clearly choose the drug which is less toxic i e cyclophosphamide

It is obvious that other factors than the body weight and age of myeloma patients are responsible for the variation in hematological side-effects after melphalan treatment Resorption and distribution of the drug may play a part as well as differences in the enzymatic repair of alkylating induced DNA damage

ACKNOWLEDGEMENTS

Professor Jan Waldenström provided us with a large part of the clinical and laboratory data. The work was supported by a grant from King Gustaf V Jubilee Fund

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Relative Value of Sternal Aspiration, Iliac Crest Biopsy and Biopsy Imprint in the Diagnosis of Secondary Cancer Involvement of the Bone Marrow

A Bjørneklett P Stavem and K Elgjo

From Hematological Section Medical Department A and Department of Pathology National Hospital of Norway University Hospital Oslo Norway

ABSTRACT Sternal aspirates, iliac crest biopsies and biopsy imprints from 31 patients with verified metastatic cancer to bone marrow were reviewed All three methods were shown to be complementary, as cancer cell deposits were detected in some cases by one procedure and missed by the others

The value of cytologic smears in the diagnosis of metastatic tumour cells in bone marrow has been found by several investigators to be considerably less than that of histologic biopsy (1 3 7 11) One recent report (8) however is in favour of the cytologic smear It has been our experience that cytology and histology are complementary in diagnosing metastatic cancer in bone marrow

We here report our findings in 31 patients with secondary cancer involvement of the bone marrow

PATIENTS AND METHODS

We have reviewed the preparations made from all patients admitted to our department during the period 1967–76 with a verified diagnosis of cancer metastases to bone marrow Disseminated cancer was the pre admission diagnosis in only 12 of the patients Medical Department A Rikshospitalet has a special section for hematology and this probably explains why more than half the patients were admitted suspected of having a primary hematological disorder (myelomatosis 5 acute myelogenic leukemia 2 myelofibrosis 1 aplastic anemia 4) Five of the patients were admitted because of hemorrhagic diathesis one because of chronic urinary tract infection and another because of protracted pneumonia The primary tumor was found in 17 patients (throat 2 thyroid 1 stomach 4 lung 3 breast 3 prostate 3 retroperitoneal neuroblastoma 1) In 14 cases a primary tumor could not be identified

The biopsies were taken from the posterior iliac crest with Stavem s needle (12) Imprint preparations were made by rolling the fresh biopsy along one or two cleaned glass slides Both imprints and sternal aspirates were air dried fixed in methanol and stained with May Grunwald & Giemsa The biopsies were fixed in Zenker s solution and formaldehyde decalcified paraffin-embedded cut and stained with hematoxylin azophloxine and saffron

The morphologic criteria for the identification of metastatic cancer cells in bone marrow smears were essentially those previously defined by Kingsley Pillers et al (10) All the cytologic preparations were reexamined and the presence of cancer cells was recorded as positive suspicious or negative The percentage of cancer cells compared with total number of nucleated cells was calculated It was noted whether the cancer cells were found predominantly in clusters or syncytia of 5 cells or more (Fig 1*a–c*) or if they were scattered on the slide (Fig 1*d*) All the biopsies were reexamined by the pathologist and recorded as positive (Fig 2*a–d*) suspicious or negative

It is our opinion that a cytologic diagnosis of cancer should whenever possible be confirmed by another technique preferably by histology Biopsy was for various reasons not performed in four of the patients The diagnoses were however confirmed at autopsy a short time later in three of them Autopsy was not performed in the fourth patient but all other investigations indicated that the diagnosis of metastatic carcinoma was correct

RESULTS

The histologic bone marrow sections were positive of metastatic cancer in 21 suspicious in two and negative in three patients In all three cases with negative biopsies cytology showed nests of metastatic cancer cells On the other hand we were unable to obtain cytologic preparations containing marrow elements from one patient Repeated sternal aspirations gave dry tap and the imprints fa

to show anything but peripheral blood In this case the biopsy was infiltrated by cancer accompanied by large amounts of fibrous tissue In seven patients sternal aspiration failed to produce adequate preparations due to dry tap In six of these cases however adequate imprint preparations were made from the iliac crest biopsy In only 14 of the cases were representative cytologic preparations of both types present In two of these 14 cases the cancer metastases failed to be diagnosed in the sternal aspirate whereas in the remaining 12 the diagnosis was made from the sternal aspirate as well as the imprint from the iliac crest The cytologic diagnosis was positive of metastatic cancer cells in 30 of the 31 patients

The cancer cells amounted to more than 50% of nucleated cells in 11 of the sternal aspirates and in 13 of the biopsy imprints In some cases normal marrow elements could hardly be identified Five of the sternal aspirates and 2 of the biopsy imprints contained 10–50% cancer cells less than 10% were found in 6 of the sternal aspirates and in 5 of the biopsy imprints In some of these cases a thorough search through the entire preparation was neces[illegible]ary to identify the cancer cells

[illegible] 12 of the sternal aspirates and 13 of the biopsy [illegible] the cancer cells were found predomi[illegible]y in clusters or syncytia (Fig 1*a–c*) In 8 of the sternal aspirates and 7 of the biopsy imprints the cancer cells presented both in clusters and as scattered cells In 2 cases the cancer cells were found only as scattered cells in the sternal aspirates (Fig 1*d*) In one of these cases the biopsy was unequivocally positive In the other case the presence of atypical cells could only be suspected in the first biopsy but a second biopsy was positive

As mentioned above 5 of our patients presented with severe bleeding disorders Biopsies were performed in all these cases with no complications This is in accordance with the experience of others (5) In one of our cases reported elsewhere (2) we were able to study the effect of treatment on the cancer cells by repeated sternal aspirations

DISCUSSION

The recognition of metastatic cancer cells in bone marrow smears requires an experienced investigator who should be well acquainted with the bone marrow cytology in various conditions i e all the primary hematological malignancies Most of our patients were referred from other hospitals and sternal aspirates had been incorrectly interpreted in several instances On the other hand there is a problem with false positives Emerson and Finkel (7) found 22 false positives in a prospective double blind study of 100 patients We have also during the actual period made some doubtful cytologic interpretations However repeated examinations and biopsies have tended to solve these problems One must be aware that clumps of normal marrow elements may closely mimic metastatic cells and atypical megacaryocytes, plasma cells osteoblasts osteoclasts endothelial cells and histiocytes have at times been confused with tumor cells (4 6) The difficulties in interpretation of tumor cell metastases are probably less on histological sections These may also in some cases allow a more accurate classification of the type of tumor cell present

No less than five of the patients in this series were admitted because of abnormal bleeding tendency In all five coagulation tests were compatible with disseminated intravascular coagulation (DIC) This should stress the importance of looking for disseminated malignancy as a cause of DIC

Grann et al (9) showed that the combination of sternal aspiration and iliac crest biopsy increased significantly the diagnostic yield in patients with metastatic carcinoma of the bone marrow We find that sternal aspiration iliac crest biopsy and biopsy imprint should all be performed as there are cases in which one procedure will detect deposits of cancer cells missed by the others

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Fig 1a

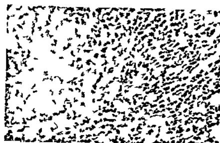


Fig 2a



Fig 1b

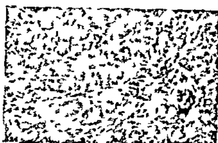


Fig b



Fig 1c



Fig ?



Fig 1d

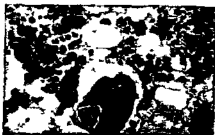


Fig 2d

Fig 1 Metastatic cancer cells in bone marrow smears from three patients May Grunwald & Giemsa (*a* and *b*) Biopsy imprint Cluster of cancer cells Primary tumor breast carcinoma *a* × 400 *b* × 1 000 (*c*) *Sternal aspirate Group of metastatic cancer cells with high* degree of cellular atypia. Primary tumor not identified × 1 000 (*d*) Sternal aspirate Two scattered cancer cells with vacuolated cytoplasm Primary tumor not identified. × 1 000

Fig 2 Sections from bone marrow biopsies from two patients showing cancer infiltration Hematoxylin azophloxine and saffron (*a* and *b*) Cancer cells in clumps sometimes showing alveolar arrangement Fibrous tissue surrounding the cancer cells Primary tumor breast carcinoma 160 (*c* and *d*) Clump of cancer cells in the bone marrow probably inside a small vein Primary tumor not identified *c* × 160 *d* × 400

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Macroglobulinaemia in an Icelandic Family

Ólafur Grimur Bjornsson [1] Alfreð Arnason Sigurður Gudmunðsson
Ólafur Jensson Snorri Ólafsson and Helgi Valdimarsson

From the Departments of Clinical Biochemistry and Medicine Landsspitalinn University Hospital of Iceland the Blood Bank of Iceland Reykjavik Iceland and the Department of Immunology St Mary s Hospital Medical School London England

ABSTRACT Macroglobulinaemia in an Icelandic family is presented A woman had Waldenstrom s macroglobulinaemia and two of her brothers had monoclonal macroglobulinaemia of the benign form One was asymptomatic but the other had poly neuropathy and IgM deposits in peripheral nerves A third brother of these siblings died of a lymphoreticular disease, which presented with a widespread neuropathy A second sister had polyclonal increase in serum IgA and two other brothers of this sibship had IgM slightly elevated A study of all descendants (45 in all and 19 spouses) revealed seven individuals with elevated IgM levels No other immunoglobulin abnormalities were detected

M-component of the IgM type has been found in 0 01–0 1% of individuals in random population studies (2 7) As with other M components the frequency of IgM paraproteinaemia increases with age (2 14) and one study of people over 65 (6) has shown a 2% frequency of IgM M-component (10/500) The prevalence of these paraproteins is higher in relatives of affected members Several authors have reported IgM M-component in 2 and rarely 3 members of the same family (8 13 16 22 23 24) These individuals were mostly asymptomatic

We report an Icelandic family of 12 members and three generations with elevated IgM levels thereof three siblings with IgM M-component One of these siblings the propositus has IgM M-component of the benign form associated with IgM and amyloid deposits in peripheral nerves and poly neuropathy

STUDY POPULATION AND METHODS

Every member of the family including 19 spouses (Fig 1) has been surveyed as described below

Quantitative estimation of IgA IgG and IgM was per formed by electroimmunoassay as described by Laurell (15) Specific antisera and control sera were purchased from DAKO-immunoglobulins Denmark Immunoglobu lin typing was performed by immunoelectrophoresis against specific antisera. C_3 and C_4 were measured by the standard single radial immunodiffusion technique Qualitative investigation of protein fractions was made by horizontal agarose electrophoresis (11) and starch gel electrophoresis (17)

Rheumatoid screening tests included Rose Waaler and latex agglutination tests acryl fixation test LD cells anti streptolysin O titer and tests for cryoglobulins

Bleeding parameters included Quick test PTTK prothrombin time (Owren) bleeding time fibrinogen de gradation product and coagulation factors V and VIII

Sections of sural nerve biopsy were tested for immunoglobulin and complement deposits by direct immunofluorescent microscopy Blood lymphocytes were tested for membrane immunoglobulins using FITC la belled $F(ab)_2$ anti human μ γ and α

CASE REPORTS

Case II 1

This 82 year-old seaman was investigated as part of this study He was symptom-free and normal on physical examination His ESR was 60 mm/h (Westergren) but his Hb and blood film were normal Total protein was 7 0 g/100 ml and electrophoresis showed a spike in the γ-region which measured 28 8% of the total protein (Table I) Immunoelectrophoresis showed IgM kappa para protein serum IgA 70 IgG 714 and IgM 2 268 mg/100 ml

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[1] *Present address* MRC Building 5th floor Gastroenterological Unit Hammersmith Hospital DuCane Road London W 12 OHS England

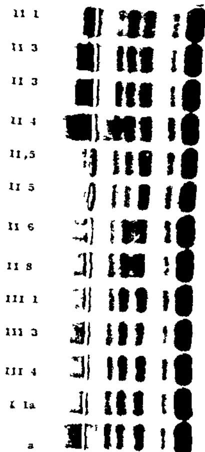


Fig 2 Agarose gel electrophoresis (pH 8 6) showing bands in γ-globulin region of cases II 1 II,3 and II,5 The two specimens from cases II.3 and II.5 are from different times There is a diffuse increase in the γ–β region of case II 4

serum (Table II Figs 2 and 3) and the fact that 84% of her bone marrow cells were lymphocytes were considered consistent with the diagnosis of Waldenstrom s macroglobulinaemia.

A treatment course with chlorambucil 6 mg daily and prednisolone 60 mg was started In Dec 1976 this treatment was withdrawn due to leukopenia (WBC 400/μl) In March 1977 she suffered from herpes zoster eruptions She has lately developed ecchymotic lesions on both fore arms and is staying in a hospital

Cases II 4 II,6 and II,8

The other two brothers alive (cases II 6 and II 8) aged 71 and 69 years respectively were healthy and had normal ESR. Both had abnormally high serum IgM levels (Table II) and case II 6 had low IgA but there was no evidence of M-component in their serum. The second sister (case II 4) aged 74 years had a polyclonal increase in IgA IgA 444 IgG 1795 and IgM 63 mg/100 ml

Cases II,7 and II 9 died at an early age from diphtheria.

Cases III,2 3 4 7 and *IV,3 13 and 23* were healthy There was no known consanguinity in this family and other members were asymptomatic as far as we know Further study on cellular immunochemical and genetic factors in this family is under way

RESULTS

The relationship of the family members is shown in Fig 1 Diseased family members are found in generation II no 2 3 and 5 and their clinical records are summarized above Fifty normal males and 50 normal females aged 20–50 years served as controls for immunoglobulin estimations and normal ranges were determined as means±2 S D

Serum protein levels and albumin/globulin ratios are shown in Table I The results of electroimmunoassay and light chain typing are shown in Tables II and III The qualitative investigation of protein fractions made by horizontal agarose electrophoresis is shown in Fig 2 and by starch gel electrophoresis in Fig 3

DISCUSSION

The occurrence of a paraproteinaemia in several members of the same family may suggest a genetic predisposition to B-cell neoplasia and abnormal

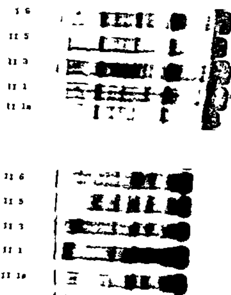


Fig 3 Starch gel electrophoresis (pH 8 6) without (top) and with 0 04 M mercaptoethanol added (bottom) Different electrophoretic mobility of bands in the γ-globulin region can be seen in patients II 1 II 3 and II,5

Table III *Immunoglobulin estimation (mg/100 ml) in seven asymptomatic family members aged 15–49 years*

Family members	Sex	IgA	IgG	IgM
III 2	♀	165	1 142	114
III,3	♀	160	1 071	136
III 4	♀	121	918	232
III 7	♂	167	1 387	128
IV 3	♀	224	1 428	146
IV 13	♀	145	989	158
IV 23	♀	44	775	124
Controls (±2 S D)		55–320	650–1 600	20–110

immunoglobulin synthesis The genetical defect remains unknown Chromosomal abnormalities found in individuals with paraproteinaemia seem not to be inherited (23) and structural gene abnormalities are not likely to be involved because different immunoglobulin classes are found in the families with paraproteinaemia (8 13 16 22 23 24)

Of the three siblings with M-component described here all have an M component of the IgM class but they differ in electrophoretic mobility and in types of their light chains Two have light chain type kappa and one has light chain lambda

Association of HLA B7 with paraproteinaemia has been reported (5) However on HLA and properdin (Bf) typing of all members of the family reported here no apparent association was found with members with elevated IgM levels (1)

Criteria for Waldenstrom s macroglobulinaemia in patient II 5 (Fig 1) and benign monoclonal macroglobulinaemia in patients II 1 and II 3 are well established (10) However no M-components have been detected in the other nine family members (Fig 1 Tables II and III) who have IgM levels above the upper limit of normal for Icelanders Six of them are women IgM has been found to be higher in females than males and its level is thought to be related to the number of X chromosomes (20) IgM has also been found to be race-dependent (12) The control group used in this study indicates that IgA and IgM levels are unusually low in Icelanders and further study on this in both sexes is under way (4)

In 1973 Forssman et al (9) first reported a patient with peripheral neuropathy and benign IgM M component In this patient IgM producing lymphocytes infiltrated the endoneurium of affected nerves These lymphocytes were eradicated and all symptoms of neuropathy disappeared after a short course of chlorambucil

The propositus reported here (patient II 3) presented at least 7 years ago with neuropathic symptoms which slowly progressed in spite of treatment with chlorambucil for 5 1/2 months However this is not surprising because his neuropathy is presumably due to IgM or amyloid deposits and not to cellular infiltration Antigen–antibody reaction between the IgM paraprotein and a nerve specific component is unlikely in our patient because amyloid material was also found in his lymph nodes and liver His serum did not contain significant rheumatoid factor activity and C3 and C4 in serum were normal However the presence of IgG in addition to IgM in the neuronal deposits and the high percentage of blood lymphocytes with both IgM and IgG in their surface membrane suggests that his monoclonal IgM may have reacted with IgG This is supported by the finding of 22S immunoglobulin fraction in serum Lymphocyte membrane bound IgM with IgG binding activity has been described in patients with Waldenstrom s macroglobulinaemia and also in chronic lymphatic leukaemia and this has not always been associated with detectable rheumatoid IgM factor in serum (18)

Patients with extensive polyneuropathy IgM M component and amyloidosis have been reported (3 21) but lack of follow up (3) and lack of exclusion of malignant diseases (21) make it disputable if these cases could be classified as monoclonal macroglobulinaemia of the benign form IgM deposition on myelin sheaths has been described in Waldenstrom s macroglobulinaemia with polyneuropathy (19)

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Relapsing Annular Erythema and Myeloma Successfully Treated with Cyclophosphamide

Gosta Krook and Jan G Waldenstrom

From the Departments of Dermatology and Medicine Malmo General Hospital Malmo Sweden

ABSTRACT We report the history of a patient, who had recurrent patches of erythema on the trunk and extremities for some years The diagnosis of erythema elevatum diutinum or annulare centrifugum was discussed The patient developed a monoclonal IgA lambda fraction and clinical signs of myeloma Treatment with cyclophosphamide stopped the development of erythema and also caused the M-component to disappear This improvement has persisted even without cytostatic treatment Another patient with prostatic carcinoma and relapsing annular erythema was cured of his skin lesions when his carcinoma was treated with estrogens The literature is reviewed

CASE REPORTS

Case 1

A woman born in 1912 Her mother has been operated upon for a carcinoid tumour and is alive and well at 84 In 1950 the patient noticed profuse sweating probably menopausal In 1968 she had a single bleeding from the uterus Biopsy was normal and regular controls have been negative

In Dec 1968 she started to have frequent diarrhoea watery stinking In Jan 1970 she had only one very loose stool every morning but no abdominal pains The patient lived under considerable psychological pressure It was noted that her ESR was 30–50 mm/1 hour

Since the spring of 1969 the patient had noticed recurrent red infiltrated patches and annular skin lesions on the elbows upper arms shoulder regions and thighs The annular lesions started with a pink infiltrated papule which slowly enlarged and formed a non itching ring as the central area flattened and faded In about two weeks the ring usually reached a diameter of 6–8 cm During the following weeks the lesions slowly regressed In Dec 1970 she was seen at the Department of Dermatology On both upper arms and at the elbows the patient had erythematous somewhat elevated and about 5 mm broad annular eruptions without scaling The largest rings were about 7 cm in diameter In the center the colour was brownish (Plate Figs *c* and *d* pat E H)

The skin lesions in our patient seem to be compatible with the clinical diagnosis of erythema annulare centrifugum A skin biopsy examined by Professor F Linell shows a normal epidermis In the upper part of the corium there are distinct histological changes with pericapillary lymphocytic infiltrates Some of the capillaries have slight hyaline changes in the wall The most evident change however is the very dense and diffusely distributed infiltrations of polymorphonuclear leucocytes There are also numerous nuclear splits

Intradermal injections of 0 1 ml of the patient s serum and plasma respectively were given in her own skin but did not provoke any skin lesion External treatment with Betnovat® ointment (betamethasone 17 valerate) with plastic occlusion or intralesional injections of Kenacort T® suspension (triamcinolone acetonide) arrested the extension of patches and rings but new large ones developed periodically during the following years in new locations

In Sept 1971 the patient was seen by one of us (J W) because it had been found that she had an immunoglobulin M-component in her plasma and the question arose whether she suffered from myeloma She had a normal hematological status although the ESR was quite high 68 mm/1 hour The condition was followed during the next year In June 1972 she had some further troubles from her colon and a proctoscopy was performed Nothing pathological was found Radiology of the skeletal system was normal

During 1972 she noted pains in her thorax on pressure A new X ray of the skeleton disclosed a large number of lytic lesions in the skull partly confluent On the right side of the thorax several ribs had small lytic lesions

Because of this finding Sendoxan 100 mg daily was given up to 1900 mg starting on Feb 3 1973 She was then put on 50 mg daily and was seen regularly Her pains disappeared very rapidly and her skin was much improved On the whole it may be said that she has had no typical erythema since about half a year after the start of the Sendoxan treatment

Table I *Electrophoresis etc (case I)*

	Hb (g/l)	ESR (mm/1 h)	Albumin (g/l)	α_1 (% of normal)	Orosomucoid (% of normal)	Haptoglobin (g/l)	Fibrinogen (g/l)	IgG (g/l)	IgM (g/l)	IgA L (g/l)
1/71			43	120	100	1 3	3 0	6	0 2	28
6/71	132	86	40	100	70	0 6	2 5	5	0 2	28
12/71	128		46							25
6/72	112		42			0 9				26
2/73	116	130	40	90	50	0 9	2 5	3	0 06	32
X ray signs of myeloma Cyclophosphamide										
4/73			47	110	70	0 6	4 0	2	0 06	17
9/73	133		47	110	80	0 9	3 2	3		4 (No M)
1/74			48	130	90	0 8	3 7	4	0 1	3
5/74			47	120	80	0 6	3 5	5	0 08	–
9/74		8								
1/75	112		46	110	80	1 1	2 9	4	0 1	–
5/75	138	9								
12/75	134		Cyclophosphamide stopped							
2/76	140	4								
6/77	139	20	43	130	110	1 0	3 0	5	0 1	4

In Dec 1974 she had more pains in her left hip but X ray examination was negative and her ESR was only 8 mm/1 hour Her condition has been quite satisfactory and there have been practically no skeletal pains She taking 50 mg Sendoxan every day and seems to have little trouble Her blood values are remaining normal -an was discontinued in the middle of Dec 1975 On Feb 6 1976 calcitonin was started in order to find out whether this might help in the healing of her lytic lesions A picture of the cranium in Nov 1975 showed no change from 1973 In June 1977 her status was still excellent with no subjective symptoms and a normal skin The electrophoretic pattern of this patient is shown in Table I

Sternal puncture in Jan 1971 showed numerous plasma cells with a histological picture of a typical myeloma There were no atypical cells and nucleoli were not seen In Jan 1975 the percentage of plasma cells was 2 5

The following patient may also be of interest in this connection

Case 2

A man born in 1899 In 1964 he had been operated upon for duodenal ulcer In 1975 he noticed red rings in the face after exposure to sunlight Later in the same year similar changes developed on the back and arms without such exposure In Jan 1976 he had big annular lesions (diameter up to 10 cm) with an elevated border of some 5 mm and some scaling on the upper part of the trunk and arms (Plate Fig *b* pat G A)

In 1975 a prostatic carcinoma was found with a medium to low degree of differentiation Since Oct 1975 he has been treated with polyestradiol phosphate 160 mg once a month Since the spring of 1976 no new dermatological lesions have developed No dysuria is present skeletal X ray shows no signs of metastasis There is a marked increase in polyclonal IgA His serum albumin is normal No anemia has been found

The diagnosis of the lesions is erythema annulare centrifugum It has been regarded as a paraneoplastic phenomenon

REVIEW OF THE LITERATURE

The dermatological diagnosis in these patients is obviously compatible with one of the multifaceted paraneoplastic erythemas even though erythema gyratum repens in its classical form with zebra like stripes of gyrate erythema was never seen It is interesting to note that several authors with experience in this field are somewhat critical of the wide variety of names given to these erythemas

Summerly (6) has published detailed descriptions of three patients one with a myeloma another with ovarian carcinoma and a third with acute leukemia who had a skin picture very much akin to that in our patients He points out that we should not make too many subdivisions among the erythemas The patient with myeloma had a very high ESR marked plasmocytosis in the bone marrow and a marked increase in gammaglobulin Bence Jones protein was present in the urine but X ray of the skeleton was normal This woman had relapsing centrifugal erythematous eruptions on the arms and neck The advancing borders were light red and elevated the central areas were scaly and pigmented Biopsy showed swelling and prolifera

tion of the capillary endothelial cells with peri vascular cuffing The second case had a fixed ery thema on the left shoulder with no itching The borders were slightly raised and had a gyrate out line This status seems to have remained constant for about one year Three weeks after the removal of a pseudomucinous cystadenoma ovarii the pa tient became and remained cured from her skin lesions This patient also had a false positive Was sermann reaction (TPI neg) that may possibly in dicate autoimmunity It did not disappear post operatively

We have found another patient in the literature published by Thivolet et al (8) The clinical diag nosis was myelosarcoma but the picture seems to have been a typical plasmocytoma She had 70% plasma cells in the bone marrow but no osteolytic foci The IgE level in the blood was high and her urine contained both albumin and Bence Jones protein Erythematous annular lesions with a diameter of 1–2 cm gave a polycyclic pattern over the abdomen arms and thighs These lesions were non elevated

Duperrat and Rappaport (1) have observed a woman of 46 with erythema elevatum diutinum and multiple myeloma In 1954 she started to have a bullous and itching eruption on the legs Later she had nodular red eruptions on the knees elbows and extensor side of the fingers These formed a red elevated ring structure and the clinical diag nosis was erythema elevatum diutinum in a typical form Biopsy showed endoarteriolitis with a dense perivascular infiltrate of polynuclear cells and some eosinophils The patient was treated with corticoids but had a number of new eruptions The lesions never disappeared completely In July 1957 she was in a poor condition with dyspnea edema and proteinuria In Nov 1958 she had a number of pig mented scars after her previous infiltrates both on the arms and the legs Serum electrophoresis was performed and she had a hyperglobulinemia of 46 g/l Immunoelectrophoresis revealed a very marked increase in IgA with low gammaglobulins In the urine she had a marked increase in gamma A globulin (Institut Pasteur) There was no hyper calcemia and no signs of renal dysfunction but she had a typical picture of myeloma on X ray ex amination of the skeleton and 12% plasma cells in the bone marrow She had never had any skele tal pains

A Congo test was performed three times and showed very markedly pathological values (65% disappearance in 2 min) with very little urinary ex cretion Hepatic biopsy could not be performed as the patient had a prolonged prothrombin time in spite of treatment with vitamin K This is a very interesting observation even though the dermato logical picture was quite different in our patient who had no bullae and showed complete reversibil ity of the skin eruptions with formation of new macules all the time until she was treated for her myeloma

The literature regarding the combination of typi cal erythema gyratum repens and malignant neo plasia has become quite extensive during the 25 years that have elapsed since Gammel (2) first de scribed this combination In a paper from 1966 Pevny (4) collected 8 cases from the literature and added one of his own The dermatological picture was very similar in all but the localization of the carcinoma varied greatly Two patients with mam mary carcinoma one with bronchial two uterine one lingual two gastric and one hypopharyngeal were known at this time Since this publication one patient with squamous cell carcinoma of the lung and typical dermatological changes that dis appeared after 2 weeks when the patient received intensive treatment with X rays has been de scribed

Thomson and Stankler (8) observed a woman of 73 who was first regarded as an instance of pityria sis rubra pilaris but later showed the typical picture of gyrate erythema This woman had a big tumour in the vesica urinaria Another patient developed the typical dermatological lesions 6 years before a prostatic carcinoma was discovered

Hochleitner et al (3) published the history of a patient who had an inoperable squamous cell car cinoma The publication contains excellent pic tures It was remarkable that the patient had pig mentations on the mucous membranes in the mouth and also showed hyperkeratosis on the hands and feet

In a number of patients the occurrence of chang ing erythema either of the gyrate type or more centrifugal has announced the presence of a tu mour that could be removed The skin symptoms then always disappeared quite rapidly It is probable that increased knowledge regarding such symptoms will become an important diagnostic sign in oncology

Many hypotheses have been presented about the

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Pyoderma Gangraenosum (Dermatitis Ulcerosa) and Monoclonal (IgA) Globulin Healed after Melphalan Treatment

Case Report and Review of the Literature

Halvor Moller Jan G Waldenstrom and Olle Zettervall

From the Departments of Dermatology and Medicine Malmo General Hospital Malmo Sweden

ABSTRACT Since 1968 we have been treating a patient who has had a combination of pyoderma gangraenosum (dermatitis ulcerosa) and signs that may indicate early multiple myeloma She also had carcinoma of the colon, which was successfully operated The pyoderma healed later after intensive and successful cytostatic treatment of the ' myeloma" The ulcers remain practically healed and the protein pattern is normal in May 1977 Such cases are rare and a search in the literature has not been very rewarding In our own series of more than 200 cases with myeloma this combination is unique The literature is discussed in detail with data on the follow up on some of the patients

It is well known that pyoderma gangraenosum (PG) may be seen in a number of gastroenterological conditions above all in ulcerative colitis We are not aware of any publication regarding a combination with a carcinoma of the colon that is not connected with ulcerative colitis Operative removal of the carcinoma in our patient did not influence the dermatological picture and we are inclined to think that this was a mere coincidence The ulcers healed during treatment of the gammapathy with melphalan A clinical picture resembling myeloma has been noted in several published cases

CASE REPORT

Woman born in 1893 Previous history of no interest In Oct 1968 an ulceration developed on the right lower leg after a trauma Biopsy showed no malignancy On Nov 7 she was admitted to the Department of Dermatology for hospital care Her general status was good with a BP of 165/80 No pathological physical symptoms apart from those from the skin The development of the dermatological symptoms is described later (Plate Fig *a* pat E L)

It was immediately found that the patient had a rather severe anemia and she was given two units of blood without any undue reactions A course of Sulfadon® (sulphaproxylin and sulphamerazin) was tried but after 10 days she developed high fever and a generalized exanthema On admission the ESR had been 70 mm/l hour Wassermann reaction was negative Serum iron 60 µg/100 ml with TIBC 300 Electrolytes and creatinine were normal Hb was 80 g/l with 2 7 mill red cells Leukocyte counts normal An electrophoresis was performed (Table I) and showed a small IgA M component In Jan 1969 a carcinoma coli was found but the patient declined operation

A general skeletal X ray survey in Sept 1969 did not disclose any signs of myeloma A repeated X ray of the colon confirmed the diagnosis of carcinoma Her general condition was fairly good but she weighed only 50 kg She had another transitory drug eruption from Mogadon®

After much discussion with the patient she consented to have her carcinoma coli operated on in April 1969 The operation seemed radical (adenocarcinoma) She was later treated with chloramphenicol The usual course of melphalan was started in Sept 1969 with a loading dose of 5 mg/day followed by 2 mg/day This was stopped on Jan 3 1970 because of increasing anemia She had also received 5 blood transfusions Sternal puncture in Feb 1969 had shown a considerable increase in large plasma cells partly of the so-called thesaurocyte type but no flaming plasma cells One plasma cell with nuclear inclusion was seen No clearcut proof of myeloma

In Aug 1970 there was continued general improvement with a body weight of 55 kg In Feb 1972 she was in very good shape with no ulcerations Hb 11 2 RBC 3 4 mill leukocytes 4700 platelets 228 000 ESR 55 mm/1 hour The patient had a course of cyclophosphamide in March 1973

When last seen in June 1977 the skin lesions were still healed she had no symptoms of myeloma but declined another sternal puncture or X ray of the skeleton (Table I)

Table I *Electrophoretic pattern and treatment*

Date	Albumin (g/l)	Hapto-globin (g/l)	α anti-trypsin (% of normal)	Oroso-mucoid (% of normal)	IgG (g/l)	IgA (g/l)	IgM (g/l)	Remarks
11/68	37	3 40	214		90			
12/68	34		227	214	60	6 0	0 5	
1/69	32				70	3 0		Steroids
3/69	32	2.55			60	4 0	0 3	Sternal puncture 2/69 Large plasma cells partly thesaurocytes No flaming. One nuclear inclusion. Skeletal X ray negative
4 69	Operation of adenocarcinoma coli							
9 69	Started on melphalan							No improvement in ulcers
1/70	Melphalan stopped							
3/70	56	1.35	260	150	60	1 0		
11/70	Melphalan resumed							
	58					No M-component		
2/72	45				80	2 0	0 6	Skin practically healed
3/73								Sendoxan course started
7/73	44		150	160	90	2 0	2 (high)	
12/73	47	1 60	120	140	90	2 2	1 6 (small IgA M-component)	Small ulcers
6/77	46	2 0	120	120	110	2 3	5 (no IgA M-component)	

Development of the dermatological symptoms

1968 following a slight trauma to the right lower leg the patient developed a non-healing purulent and hemor- It measured 4×5 cm and was situated medially on the lower third of the lower leg. The surface was bright red and exudative partially covered with purulent or necrotic crusts. The border was irregular with cyanotic nodules and an outer light red halo The histologic picture in repeated biopsies was that of a purulent partially necrotic inflammation with some vascular proliferation but no vasculitis. Bacteriologic cultures were initially negative

During the following year the ulcer extended to most of the right lower leg and similar lesions developed on the left leg (Plate Fig. *a*) The PG was only slightly improved by prednisolone up to 60 mg a day in 1968 and up to 30 mg a day in 1969 It was not at all influenced by surgical removal of the colonic carcinoma in April 1969 From Sept 1969 concomitantly with her melphalan treatment, the patient s ulcerations started to heal leaving a pinkish induration, sometimes with hypopyon-like micropustules or hemorrhagic dots. Except for a limited relapse on the right lower leg in 1970 the PG has been healed at later examinations most recently in June 1977

REVIEW OF THE LITERATURE

A large number of observations have been published regarding the simultaneous occurrence in plasma of PG and monoclonal gammaglobulins *usually of the IgA type* but the connection with clearcut myeloma is more difficult to establish

*It is possible that some old publications regard*ing chronic ulcers in myeloma patients are really instances of PG No firm diagnosis can be made however from the available descriptions of the dermatological picture Cowan (2) published a *paper in 1961 on a man who started to have papules* that ulcerated and then healed on his upper chest and back

The lesions recurred frequently ulcers in the flank and on the arm did not heal The published picture closely resembles PG This patient had massive Bence Jones proteinuria and an intense myeloma-like band in the α_2 region. The background γ-globulin was quite low the skeleton was normal radiologically but the bone marrow contained 30–50 % plasma cells This is practically sure proof that he had an IgA myeloma. The myeloma was not treated

In 1962 Duperrat et al (6) presented a patient who had a skin condition which seemed to be PG Because of this diagnosis some possible causes were investigated and serum electrophoresis showed that there was a low gammaglobulin and a high beta fraction (25 g/l) Immunologically this was an IgA fraction There was no excretion of Bence Jones protein and no skeletal findings Sternal puncture was normal but the fact that the patient also had a low gammaglobulin makes the diagnosis of myeloma very probable

Rockl et al (11) published a paper in 1964 on four patients with PG and M components of IgA type Three of them certainly did not have any myeloma whereas it was considered possible that the fourth suffered from multiple myeloma Two other patients had no visible M component but this does not mean that none was present It is not rare for small M components to escape notice when immunoelectrophoresis is not performed Schropl (12) from the same department published in 1967 an other male patient (52 years old) with PG IgA and no clinical signs of myeloma

Some years later v d Sluis (13) described two patients who also had monoclonal IgA

The first was a man aged 56 who had a small ulcer on the left leg This became larger and new ulcers developed on the trunk and left elbow IgA was increased and was regarded as monoclonal The IgG was low There was no excretion of Bence Jones protein Bone marrow and osseous system were normal During the next 4 years he had ulcers continuously At reexamination immunoelec trophoresis gave the same result but this time the patient had excretion of Bence Jones protein The author does not make any firm diagnosis but it seems to us that this was probably a patient with early myeloma The second case was a man born in 1909 In 1963 he had some ulcers on the right lower leg The clinical picture was not quite like the first one but he had relapsing ulcers It is probable that this man also had a monoclonal IgA Skeletal X ray and bone marrow examination gave no arguments in favour of the diagnosis of myeloma

Dr Imhof has informed us of these two patients published by van der Sluis in 1966 One of the pa tients was lost for follow up

The other patient who had a very serious pyoderma from 1960 onwards was treated with various methods but not with cytostatics In 1965 he was again admitted to the hospital with severe dermatological symptoms He died from uremia with hyperkalemia. At the post mortem the cause of death was found to be *general amyloidosis* in liver and spleen The kidneys were severely damaged practically all glomeruli were destroyed by amyloid There were no changes in the bone marrow It is difficult to decide whether this is amyloid secondary to the long drawn suppurating ulcerations or primary amyloidosis

In 1967 Meiers et al (10) described a woman of 50 who had had extensive ulcerations for 17 years

The picture was interpreted as a PG There were no signs of ulcerative colitis nor of myeloma The patient died from pulmonary embolism and the anatomical diag nosis of the skin lesions became vasculitis She had a rudimentary IgA M component The patient also had diabetes and polyneuropathy The data are too scanty for a clearcut diagnosis but it seems more probable that this was an instance of Ig complex forming disease

In the same year Jablonska et al (8) gave an excellent account of two cases with typical PG and myeloma

The latter disease occurred in their two patients after 2½ and 6 years respectively following the development of the skin lesion Both cases were of IgA type The authors point out the importance of this skin disease as an indicator of myeloma According to Jablonska et al Her mer Abgrans has seen a patient with PG and a myeloma of IgA type who did not have any skeletal lesions but a very marked infiltration of the bone marrow Marcussen was probably the first to stress the presence of hypogamma globulinemia in PG Some later authors have also noticed increases in β-globulin without being able to analyze the conditions in detail Some of the following case histories show that IgA is not the only type of immunoglobulin present in monoclonal form together with this condition

Degos et al (5) in 1966 described a man of 68 who had typical PG that was very refractory to treatment *The analysis of the serum is difficult* to judge as the description is very vague but the author maintains that the patient had a gamma G protein

Imhof et al (7) observed a woman of 60

In the summer of 1966 she noticed an ulcer on the leg which did not heal Later she developed several ulcers on both legs that were typical for PG Immunoelectrophoresis showed *an IgG kappa subtype 3* The normal immuno globulins were low There was no urinary Bence Jones Skeletal X ray was normal and sternal puncture showed nothing pathological During the next year there was no progression and the skin lesion healed slowly with pig mentation Dr Imhof has informed us about the continued development in this patient In Oct 1968 X ray of the skeleton and sternal puncture showed nothing remarkable In July 1969 she was admitted because of nausea and vomiting Her liver was enlarged The ulcerations on the legs were healed She now had marked anemia Hb 6 0 RBC 2 0 ESR 158 mm/l hour The differential count showed 80% plasma cells 6000 WBC Sternal marrow showed typical myeloma and she also had small osteolytic bone lesions There was no Bence Jones proteinuria Alkeran therapy was therefore started During the next period a palpable tumor in the abdomen was found but the patient developed a septic condition with irreversible shock and died 48 hours after the beginning of the fever period No permission was obtained for a post mortem This seems to be a very interesting example of a patient who had a constant IgG M-component for 4 years Her PG healed and remained well for 4 years and did not re lapse when the patient developed clearcut symptoms of myeloma with plasma cell leukemia

Another patient—with a monoclonal macroglobu lin—has been described by Cream (3) in 1971

A man of 73 who since 1950 had had multiple abscesses that developed into ulcers healing very slowly with scar

ring In 1956 an M-component with a mobility between β and γ was discovered He was not admitted for closer investigation until 1969 He then had a typical picture of PG His ESR was only 14 mm/l hour There was no anemia and the differential count was normal Serum albumin was 3 9 and globulin 4 1 g/100 ml Quantitative immunological examination of his serum showed IgG 0 8 (regarded as low) IgA 0 27 (normal) IgM kappa 0 6 (considerable increase) No Bence Jones protein could be discovered in the urine concentrated 40 times The erythrocytes showed clumping but no real cryoprecipitate It seems probable that the red cell clumping on counting may have been connected with precipitation of an euglobulin It is very hard to judge the real importance of this red cell agglomeration

In a paper treating the problem of monoclonal immunoglobulins in other conditions than myeloma Danon et al (4) mention three patients with PG without giving any information about the type of heavy chains

Kovary et al (9) described a patient with the typical dermatological condition

She had a plasma cell count of 9-15% of the white cells in the bone marrow This indicates a massive plasmocytosis There was no evidence of myeloma on X ray of the skeleton She had an IgA kappa protein in the serum of ut 1 g/l The patient was treated with rather large doses ednisone and the skin condition healed in 2 months M component was unchanged

DISCUSSION

Our present observation indicates that treatment of the patient with cytostatics resulted in almost total disappearance of an IgA M component together with healing of the PG It is of course difficult to draw any firm conclusions from one case where the combination may of course be fortuitous It is interesting that the group of patients suffering from PG and with reliable analysis of the serum proteins should show such a high incidence of IgA On the other hand it is clear that one case with monoclonal IgG and possibly another with IgM were found The fact that other (normal) Ig fractions have been depressed may cause decreased resistance to infections

Long term follow up is necessary in order to judge final outcome The patient observed by Imhof et al (7) developed clearcut myeloma after 3 years observation time One patient observed by v d Sluis (13) died with generalized amyloidosis but had no signs of myeloma

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Osteosclerotic "Plasmocytoma" with Polyneuropathy, Hypertrichosis and Diabetes

Jan G Waldenstrom Agneta Adner Karl Gydell and Olle Zettervall

From the Departments of Medicine Malmo General Hospital Malmo and Kalmar Central Hospital Kalmar Sweden

ABSTRACT The combination of osteosclerosis, polyneuropathy, monoclonal immunoglobulin, hypertrichosis, serositis and a number of other symptoms is described It seems probable that this is a special type of myeloma Similar cases have been described in Japan and our findings are compared with the Japanese picture The age of the patients is unusually low The M-component in the plasma is small There is very little Bence Jones protein in the urine and osteolytic lesions in the skull *do not seem to* have occurred The polyneuropathy may improve during treatment with cytostatic drugs

One of the most interesting variants of myeloma is the combination of severe polyneuropathy with osteosclerosis but otherwise classical signs of myeloma We saw a patient in 1955, who had this picture His unusual skeletal symptoms were discussed by Odelberg Johnson (6) with excellent X ray pictures of the bones Later one of us (J W) has seen two cases one in consultation and a limited number of such case histories has been published in recent years (9 10) It is clear that this condition is extremely rare

A short history of the following case has been published previously (9) but the follow up of this patient seems to be of interest and will be discussed here A number of recent publications from Japan have treated this subject and the results will be quoted later in this paper

CASE REPORTS

Case I (E P woman born in 1920)

Previous history of no special interest The patient has given birth to two children without obstetric complications Menopause in 1967 Around this time she noticed a swelling of the proximal part of the right clavicle She was admitted to her local hospital and biopsy there showed the typical picture of plasmocytoma At this time cranium vertebral column and pelvis were normal roentgenologically Electrophoresis see below A sternal puncture was performed and showed a normal marrow ESR was 5 mm/1 hour and the serum electrophoresis was regarded as normal The tumour was treated radiologically In Sept 1968 she started to have paraesthesiae in the legs and feet and she also had *nightly pains She felt* better when walking around Somewhat later she complained of swelling in both knees In Nov she had much more trouble especially from the right leg and she also noted marked swelling of the feet She could not wear her own shoes

During the autumn she also noticed the growth of long black hair below both patellae and about 10 cm down the tibia At the same time she developed hirsuties on arms and cheeks She observed some decrease in muscular power in both legs especially the right Around Christmas her hands became stiff and she felt alternately cold and hot She also had noted some paresis and decreased sensitivity in the fingers In Jan 1969 she became confined to a wheel-chair as even the muscles in the trunk had become paretic

She was admitted to Kalmar Central Hospital where *several sternal punctures* were performed with dry taps Marrow obtained from the crista showed presence of possibly pathological plasma cells In the third and fourth lumbar vertebrae X ray examination revealed some rather unremarkable sclerotic changes and in the left half of the sacrum there were osteolytic changes that might indicate plasmocytoma Further radiology see below ESR was then normal 4-10 mm and electrophoresis showed nothing pathological Heller's test for urinary protein was negative The patient had no anaemia or glucosuria

She was admitted to Malmo General Hospital on Feb 13 1969 The legs were swollen below the knees The hirsuties were unchanged (Fig 1) There were no signs of interest from the internal organs apart from a slight *systolic murmur with the* maximum over the aorta BP was 180/110 From the nervous system it was noted that the function of the cranial nerves was completely normal She had pareses in all extremities but especially in the

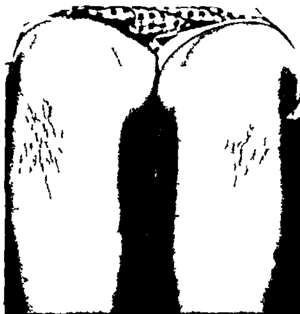


Fig 1 Case 1

legs and the right arm There was possibly some atrophy of the thenar muscles on both sides but no atrophy in the The patient was not able to lift her feet There were visible fasciculations (well nourished) Reflexes on arms were normal except that the triceps reflex was sent No abdominal patellar or Achilles reflexes could be elicited Babinski test gave no result S gns of ataxia in the upper extremities were pathological probably owing to pareses Sensibility on the upper extremities noth ng defin tely pathological regarding pain and touch Distally from the knees no sensitivity for pain and touch Passive movement of the toes was not perceived

The patient was treated with Melphalan accord ng to the usual pattern with 5 mg every day for 21 days There was no reaction from the normal bone marrow Blood chemistry was on the whole completely normal except electrophoresis of serum protein The first *electrophoresis* showed a completely normal picture and strongly con centrated urine gave only a faint albumin band When the serum was invest gated with immunoelectrophoresis it was found that it contained a small (0 4 g/100 ml) IgA lambda component in the region of transferrin and beta 1 C globul n These proteins were obviously h d ng the M-component in class cal electrophoresis The content of IgG was normal Lumbar puncture showed a marked ncrease in prote n (total 226 mg/100 ml) and the pattern was very similar to that found in the serum except that some high molecular proteins had not penetrated into the CSF Treatment with Melphalan 2 mg daily was taken up after a pause of 2 weeks and continued

The patient was sent back to her local hospital in March 1969 Her pareses were then unchanged and her sensitivity had not improved The reflexes on the arms were now absent too Complete exam nation of her coagulation status was normal There was no abnormal serology studied with a battery of tests but it was noted that there was no real depression of the common titers as is so often seen in myeloma The passive haemag glutination test for thyroglobulin was pos tive in a d lution of 1/?50 which is a borderline value and an antinuclear factor test was also borderline

Radiology of the osseous system showed sclerot c changes in the thoracic and lumbar vertebrae and some scleros s in the pelvis where also some osteolytic les ons were found A suspect lytic lesion was found in the pos terior part of L IV In the cran um there were no destruc tions In the thorax costae 5 and 6 dx showed some broadening and associated hazel nut sized destructions corresponding to the posterior axillary l ne In the right femur there was a large destruction in the upper part of the shaft (length 9 cm breadth 3 cm) The cort cal bone was partly eroded on the ins de (Fig 2)

An electroencephalogram revealed d ffuse partly ep sodic theta activity w th slight dominance of the left side

The continued treatment of the patient was very long drawn The osteolytic process in the right femur was ir radiated and Melphalan treatment in a dose of 2 mg every day was continued The patient was in an excellent mood and very cooperative regarding phys cal treatment. In July 1969 she came home and was cared for by her daughter She could not dress herself but was able to eat unaided

During the next few years she was treated partly as an out patient partly in the wards in Kalmar Central Hosp tal Continued improvement was noted In Jan 1972 she was able to walk with one stick but st ll had a drop-foot She still had an extensor defect in the fingers but was able to flex the fingers with some force In June 1972 X ray of the skeleton showed about the same p c ture In June 1973 she had no remarkable pains and took less analges cs She was put on Melphalan 2 mg every other day and th s caused some sl ght anaem a Hb 109 g/l RBC 3 7 mill haematocrit 38% WBC 2800/mm³ platelets 197000/mm³ In Jan 1975 her status was on the whole unchanged She was able to walk rather well w th her b lateral bandage for the drop-foot using one stick

In Feb 1975 she was readmitted for further phys cal therapy The pat ent had noted that the long hairs on both legs had started to d sappear in the last half of 1969 and were completely gone in 1970 Also the h rsutism on her arms and face had d sappeared She was now able to get along very well in her home with some help from the family The reflexes on the arms were present but there were no patellar reflexes No right Ach lles reflex could be el cited the left was present

In May 1977 the pat ent was readm tted because of relaps ng febrile ep sodes during the past w nter and n creasing dyspnoea and oedema of the legs Exam nation d sclosed asc tes and b lateral pleural flu d The patient is now qu te cachectic with a b g abdomen but no b o- chem cal or histological indications of liver damage Hae matology normal ESR 21 mm/1 hour (Westergren) She has now developed a renal dysfunct on with urea 15 mmol/l and creatin ne 130 µmol/l No Bence Jones prote nuria Serum electrolytes were normal X ray of the skeleton showed some progression of the sclerosis but also a large



Fig 2 Case 1 Osteosclerosis and lysis

destruction in the left part of the os ileum The large lesion in the hip showed signs of healing Several determinations of calcitonin indicated a constant slight elevation above the normal upper limit

Serum proteins have been followed during the years At the first electrophoresis in Jan 1969 nothing definitely pathological was discovered and ESR was 4–10 mm/1 hour A renewed electrophoretic examination in Feb 1969 showed albumin 40 IgG 10 g/l α 2 macroglobulin 152 mg/100 ml antitrypsin 180% (abnormal) haptoglobin 130% ceruloplasmin 20 and no visible M-component With ordinary tests the urine contained no albumin or Bence Jones and electrophoresis of strongly concentrated urine gave no Bence Jones band Immunoelectrophoresis of the serum showed an IgA lambda M-component that had obviously been hidden in the transferrin zone The whole IgA fraction is 6 g/l and the monoclonal component 4 g/l Electrophoresis of concentrated CSF showed that the majority of the proteins except the high molecular fractions had passed the barrier

In June 1970 a small M-component of 3 g/l was found IgG 9 IgM 0 9 (normal) albumin α 1 antitrypsin orosomucoid haptoglobin ceruloplasmin were normal α 2 macroglobulin was slightly low In Oct 1974 electrophoresis of the serum showed a normal IgG and IgM whereas the total IgA was somewhat high 5 g/l It is thus clear that her protein picture has remained the same during the years This is also true of the findings in June 1977

The recent developments still defy interpretation Pleural effusion has been noted in the Japanese syndrome (vide infra) No signs of carcinoma have been found The next history seems to illustrate the same syndrome without polyneuropathy

In summary it may be said that this is a patient who for several years had a solitary myeloma of the clavicle She then developed a severe polyneuropathy of the Guillain Barre type and a mixture of osteosclerotic and osteolytic lesions of the type that is seen in this form of myeloma A peculiar finding is the increased hairiness in the face on the arms and especially on the legs below the patella With radiological therapy for a large osteolytic lesion in the femur and continued Melphalan treatment during all these 7 years the patient was for several years in comparatively good shape It is remarkable that the hairs disappeared during the first Melphalan treatment indicating that they may be a secondary symptom of the disease

Case 2 (E N woman born in 1897)

We have for many years observed this patient who also had osteosclerotic myeloma She had no signs of polyneuropathy but it is interesting that she developed bilateral pleural effusions This is a symptom that may possibly be part of the syndrome

Heredity nothing of interest No previous maladies except some minor gynaecological troubles in Sept 1958 ESR at that time 16 mm/1 hour Since 1958 she was very tired and dyspnoeic In 1959 pains in the lower back and right hip X ray did not reveal anything pathological In March 1960 high fever and cough Admitted to the Hospital for Infectious Diseases with leukopenia (2 200) and an ESR of 18 mm/1 hour Routine electrophoresis showed a monoclonal component in the gamma region X rays of vertebrae pelvis and skull were normal Admitted to the Department of Medicine on March 22 Physical examination normal Sternal puncture showed 31 plasma cells/1 000 A routine determination of antistreptolysin gave a very high unexplained value BP was at that time 155/95 During continued controls the patient was found to have marked hypertension 220/120 mmHg and K W 1–2 in the ocular fundi She was not anaemic In 1964 her ESR had increased to 40 mm/1 hour She was somewhat depressed but reacted well on Tryptizol* Her hypertension was treated but did not improve very much In March 1964 her plasma cells had increased to 15% and her M-component had risen to 29 g/l Her antistreptolysin (AST) titer was at this time 1/80 000 The patient did not suffer from any severe pain and a skeletal X ray was normal She was slightly anaemic with 3 4 mill red cells

In June 1966 she had noticed oedema in the face and on the lower legs She had also become more dyspnoeic It was found that she had bilateral pleural fluid and was still hypertensive Slight febrility but no leukocytosis AST activity was demonstrated in the M-component The patient was given diuretics and improved Very faint proteinuria on two occasions otherwise normal The M-component was studied biochemically by one of the authors (O Z.)

The serum albumin remained low 28 g/l but there was no sign of protein loss in the gastrointestinal tract During the following months she had increasing anaemia. The pleural fluid was examined and showed a very high protein content 4 6 and 4 7 g/l on two occasions Total serum protein was 7.3 g/l Cytologically nothing remarkable Streptococcal hyaluronidase in plasma was normal in spite of maximal AST Her hypertension normalized during treatment but her AST titer remained very high The general condition improved considerably and the oedema decreased Because of her anaemia she had some transfusions

In Oct 1967 a new skeletal X ray disclosed sclerotic foci in many bones Reexamination of earlier X ray pictures showed that the foci had been present already in Dec 1966 On this occasion a lump was palpated in the left mamma with some palpable glands in the axilla. Ablatio mammae and exaeresis of the axilla was performed for cancer The last sternal puncture had only given a dry tap During anaesthesia for the cancer operation the surgeons therefore took a biopsy specimen from crista ilei but this did not give any positive informa The patient had postoperative radiation and during following year she was in an excellent condition subtively but her anaemia had increased and her ESR remained high (100 mm/1 hour) Her M-component had increased to 30 g/l and she had a small amount of Bence Jones protein in the urine

In Oct 1969 the patient had more pain from the right hip and a big lytic focus was found It was impossible to decide whether this was a myeloma or possibly a metastasis from her breast cancer The further development of her disease showed anaemia as before and the AST was always very high Her general condition deteriorated She needed more transfusions In Feb 1970 numerous rounded lytic foci were found in the skull and could well be caused by myeloma Treatment with Melphalan was started and was then changed to cyclophosphamide The osteosclerosis increased continuously together with the osteolytic regions in femora tibiae humeri and radii In spite of continued cyclophosphamide treatment the patient became increasingly cachectic and her severe anaemia needed repeated transfusions Her hypertension was well controlled

In 1971 her general condition was better but her erythrocytic values decreased and she had much pain periodically The patient expired on Jan 14 1972 The post mortem showed generalized myelomatosis with osteosclerosis There were plasma cell infiltrates in the adrenals but no metastases from the cancer anywhere

The following is a summary of the development of the disease in the patient previously published by Odelberg Johnson (6)

Case 3

A man born in 1905 Previous history of no special interest Around Christmas 1954 the patient developed pains in the feet with paraesthesiae Some decrease in muscular power was also noticed The patient had increased thirst and nycturia and lost 5 kg in weight. He had also noticed increased sweating and felt very hot at night Tachycardia and emotional instability were also noticed and he felt very tired

Admitted on Feb 2 1955 to the Medical Department Malmö General Hospital Nothing was noted regarding gynaecomastia. Neurologically he had *some pareses in the legs* and *decreased sensibility in both feet* His gait was somewhat hesitant Lumbar puncture showed a total protein of 162 mg/100 ml During the first weeks there was some improvement and his CSF then contained only 90 mg/100 ml protein and no cells In May he had to be readmitted because of a relapse having worked normally for some months On admission he had the same symptoms from the upper extremities and his pareses had increased His CSF now contained 172 mg/100 ml protein During the next month there was a slight improvement. In Sept he started to have *slight glucosuria* This symptom had not been noted before even during a period of treatment with ACTH His diabetic condition was treated with moderate doses of insulin which were tapered off after 3 weeks when the urine remained sugar free and he had an almost normal fasting blood sugar Initially the blood sugar had been around 200–300 mg/100 ml In Sept it was again noticed that the patient had an increased thirst with *much sweating* He maintained that he was drinking 3–5 l more than usual A renewed lumbar puncture showed a total protein of 230 mg/100 ml The electrophoretic pattern in serum and CSF was exactly the same except that α 2 macroglobulin was not seen in the urine owing to its high molecular weight

The continued course was down hill His diabetic episode did not recur the sweating had disappeared but his pareses had developed into *almost complete paralysis* Steroid treatment did not give any real improvement but it was thought that the spine should be examined radiologically after the steroid period A number of vertebral bodies especially Th XI showed a sclerotic process Also in the pelvis foci interpreted as sclerotic metastases were found but no changes in the cervical spine or the cranium nor clinical or biochemical signs of prostatic carcinoma The presence of an M-component in the electrophoretic diagram gave rise to the suspicion of a myeloma. Puncture of the sternum and the crista iliaca showed some increase in plasma cells During the last weeks of his life the patient was almost completely paralysed He had no anaemia, however but a slight leucocytosis Attempts to treat the myeloma with urethane had no effect

The patient died on Oct 10 1956 The findings at the post mortem were compatible with the diagnosis of myeloma. The osteosclerotic foci in the vertebrae showed rather coarse trabeculae and small lacunae completely filled with plasma cells There was a diffuse spread of plasma cells in the bone marrow but no tumour like plasmocytomas

Electrophoresis Already on admission in Feb 1955 it

was noted that the total gamma fraction was increased to 13 g/l with a very faint slow M-component The same picture was seen throughout his disease but at this time immunological typing was not yet possible The slow moving protein penetrated the gel and had a low carbohydrate content definitely indicating that it was an IgG

In summary it can be said that this was a middle aged man who developed typical Guillain Barre like symptoms that were refractory to treatment Already at the beginning a small very slowly moving M component was found on serum electrophoresis The level remained more or less constant during the course of the disease At a late stage sclerotic foci were noted but these could be observed on reexamination of the early X rays The osteosclerosis was progressive Bone marrow puncture disclosed abnormal plasma cells Transient glucosuria was noted and the patient complained of profuse sweating

DISCUSSION

In 1964 one of the authors (J W) discussed two personal observations and some publications in the literature that might indicate that this was a special syndrome (quoted by Lord Brain in 1970) In the same year Aguayo et al (1) published the history of a patient with multiple myeloma polyneuropathy and osteosclerotic lesions and since then a number of reports on such patients have appeared The monograph by Waldenstrom (10) contains a table on nine patients with this combination and one with an unexplained encephalopathy It was pointed out that all these patients had consistently very low levels of the M component globulin a picture of sclerosis of the bones and a Guillain Barre like syndrome with a high protein content in the CSF Electrophoresis showed exactly the same picture as was seen in the serum except that α 2 macroglobulin did not penetrate from the serum The patients were surprisingly young Since that time a number of patients with this triad have been described and it seems as if the points stressed by Waldenstrom are characteristic with one exception as there have been several patients who have a combination of both lysis and sclerosis in their bones No patients however seem to have had the typical punched out lesions in the cranium

In our material of about 200 carefully examined patients with myeloma only one had osteosclerosis and polyneuropathy This applies to a consecutive series from 1950–70 in the city of Malmo probably representing almost every case in this city One patient was sent to us because of these symptoms This must mean that the syndrome is quite rare in Sweden

In recent years Japanese authors have been concerned about a similar very interesting and more complex syndrome It was first described in Japanese in 1970 and 1971 as single case histories Takatsuki et al (7) regarded it as a new syndrome in 1973 These patients have plasmocytoma a small amount of IgG lambda M component focal sclerotic bone lesions and a slight increase in the bone marrow plasma cells The polyneuropathy is regarded as chronic and progressive some patients have endocrine disturbances including diabetes mellitus gynaecomastia pigmentations thickening of the skin hirsutism and a few patients also show enlargement of liver and spleen In 1974 Imawari et al (5) described a man aged 42 who had these symptoms from the nervous system and also pigmentation and thickening of the skin hirsutism in the lower extremities painful gynaecomastia and excessive perspiration He had no changes in the bones This patient was very carefully investigated from an endocrinological point of view and his total urinary oestrogens were highly increased whereas his total urinary gonadotropins were very low His testosterone in the blood was low but his serum oestradiol was high Serum electrophoresis showed no M-component on cellulose acetate A very small slow component was noted on agarose gel Total IgG was 1 490 IgA 275 IgM 124 mg/100 ml The immunoelectrophoresis suggested the presence of an IgG lambda M component Total protein in the CSF was 295 mg/100 ml X rays of the bones did not show anything remarkable There was no anaemia and the bone marrow contained only 0 8% plasma cells

In the paper quoted the authors have collected six cases from the Japanese literature Five were males and one was female All patients had an IgG lambda M component that was not very large The plasma cells in the bone marrow were not increased or borderline (one with 4%) The female patient had no bone lesions but three of the males had sclerolytic bone lesions Gynaecomastia was present in all the males Hypertrichosis was present in the female patient and four males diabetes in the female and three male patients This was also true of excessive perspiration The syndrome may be caused by remote effects of plasmocytoma via the M component The M components are probably not identical and IgG lambda components have been reported all over the world but so far these endocrine symptoms have only been found in Japanese patients

At the International Congress of Hematology in Kyoto Japan Sept 1976 Dr Takatsuki and three other Japanese colleagues reported no less than 32 cases of the Japanese syndrome They point out that there is a male preponderance of 25/27 Endocrinologically they have noted elevated plasma and urinary oestrogens with gynaecomastia and testicular atrophy A tendency to develop glycosuria was also present The M-component was always of lambda type IgG in 14 IgA in 7 and also Bence Jones protein in one

It may well be asked whether there is myeloma with osteosclerosis without a polyneuropathy? Patients with very marked and widespread osteosclerosis have been reported for instance by Wiedermann et al (11) two men 49 and 64 years old and by Engels et al (3) 3 male patients 36 46 and 65 years old who also had X ray pictures resembling those seen in our cases The first had a small abnormal peak and a typical post mortem finding. The second had widespread osteosclerosis Sternal puncture showed 35% plasma cells and para amyloid deposits were found in the ribs The third patient had some osteosclerosis The man aged 36 ered from constrictive pericarditis and autopsy ealed "myelomatous involvement of the epicarm with a pericardial effusion of 1500 cc The final histologic diagnosis was plasma cell myeloma The 46-year-old man had large para amyloid deposits The 65-year-old man had a granular osteoporosis of the ribs with fusiform and cystic swelling The vertebrae and skull appeared sclerotic Autopsy showed myeloma. Nothing was mentioned about gynaecomastia Other cases without polyneuropathy have been published (2) Our case 2 is probably such a patient

There are several problems that must be discussed

1 *Is this syndrome really a myeloma?* The very interesting studies by the Japanese authors seem to indicate that there is a group of patients with another more complicated combination of symptoms even if some of their patients have the same characteristics as the more oligosymptomatic patients in Europe and the US One of the crucial points is of course the final outcome and it seems to us as if the majority of these patients have died from their neurological complications and not from the myeloma"

Our case 1 has had a myeloma for 10 years and osteolytic lesions for 7 years She is still in excellent shape regarding her myeloma but it must be remembered that she has been treated continuously with small doses of melphalan

2 *Why is there a combination between osteosclerosis and polyneuropathy?* Nobody can offer any explanation except the assumption that special characteristics of the myeloma globulin may be responsible for both these symptoms It is interesting that the Japanese authors stressed the point that all their cases had an IgG of lambda type Our first case has an M-component of lambda type but the molecule is IgA. In all patients there has been little change in the level of the myeloma globulin during the years and this has been low except in one of the Japanese cases in whom it was somewhat more increased Electrophoretic strips may indicate that the background gammaglobulin was obviously very low One patient has had only Bence Jones proteinuria Also in our case 1 the IgA M-component was small 6 g/l and it seems evident that there is an overrepresentation of cases with unusually low levels of myeloma protein of the lambda type It has been difficult to establish whether other cases in the literature have had lambda type immunoglobulins

3 *Is there a real connection between the myeloma and the process in the nervous system?* The fact that several patients have improved as far as their polyneuropathy is concerned does not prove anything in this connection It is well known that patients with the Guillain Barre syndrome usually improve considerably if they do not die from their disease

The fact that there is slow improvement during continued treatment of the myeloma is therefore no real proof The influence of treatment on the myeloma has been difficult to follow as the usual quantitative parameters size of M-components percentage of plasma cells in the bone marrow and extent of lytic lesions in the skeleton are not very remarkable from the beginning It is evident however that our patient 1 has been a success as far as myeloma treatment is concerned even though a remission of 10 years is nothing extraordinary We would regard the fact that a few patients have had lytic lesions beside the sclerotic as indicating the correctness of the diagnosis of myeloma

During recent years observations on two patients have been published from the Johns Hopkins University (2) indicating that the neuropathy of mye

loma may disappear rather quickly after active treatment of the tumour

The first patient was a man of 46 For 2 years he had had weakness and loss of sensation in all extremities Treatment with steroids had been of no help and on admission he was bedridden He had pains and paresis in hands feet and legs The legs were more affected than the arms and the pareses were chiefly of a distal type There was no Bence Jones protein but IgG was increased The patient had a multiloculated cystic lesion in the right acromion but no other bone symptoms Biopsy showed plasmocytoma but the bone marrow from sternal puncture was normal The patient was irradiated locally with a total dose of 4000 rads A gradual disappearance of the burning sensations started shortly afterwards Half a year later he could walk unaided Two years later there was no recurrence of plasmocytoma and his neurologic condition was almost normal He was able to return to work It is interesting that the myeloma globulin was still seen on immunoelectrophoresis After 10 years he was still in good shape with no recurrence of the plasmocytoma

The second patient was 49 years old He had had numbness and tingling in feet and hands for 2 years and showed a bilateral foot drop His CSF protein was 225 mg/100 ml No cells were found He was treated with large doses of steroid and developed an iatrogenic Cushing syndrome A lytic lesion with sclerotic margins was found in the left humerus Biopsy showed typical myeloma but the sternal marrow was normal There was no amyloid in the skeleton or the rectal mucosa Electrophoresis showed an increase in IgG (2 1 g/100 ml) but no Bence Jones He was irradiated locally (3700 rads) with improvement after 3 months and practical remission of motor power after 9 months His impotence disappeared and the serum immunoelectrophoresis became normal

The paper by Davis and Drachman (2) contains a review of 30 cases of myeloma with polyneuropathy and a discussion of the results of treatment No treatment one improved 3 not improved Radiotherapy 3 improved 2 not improved Steroids 0 improved 6 not improved Antimetabolites 1 improved 10 not improved The average age of the patients was rather low (52 years) 75% were males About 80% had elevated CSF protein 55% of these patients had osteosclerotic infiltrates The authors believe that this is a remote effect of the myeloma and this would mean that the condition should be accepted as paraneoplastic

In summary it may be said that patients with myeloma characterized by a strong preponderance of males (3/1) a low myeloma globulin level with preponderance of lambda types low content of plasma cells in the bone marrow and a comparatively low age may or may not have osteosclerosis alone or combined with lysis when they suffer from polyneuropathy

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Smouldering Acute Myelogenous Leukemia

Stein A Evensen and Per Stavem

From Medical Department A Hematology Section Rikshospitalet Oslo Norway

ABSTRACT Among 195 patients with variants of acute myelogenous leukemia (AML), a minimum of 11 met our criteria of smouldering AML patients with less than 30% of blast cells plus promyelocytes in the bone marrow at the time of diagnosis who were observed without specific antileukemic therapy for a period of at least 6 months without entering a fulminant stage of the disease These patients were older than other patients with AML, they had initially relatively few infections, bled rarely, and did not enter the fullblown clinical picture typical of acute leukemia until the last months of life For these 11 patients the median survival time was 29 months from the time of diagnosis Patients with smouldering leukemia shall be observed carefully and not be given specific antileukemic therapy, at least not before they flare into a blast crisis Transfusions, antibiotics and a small dose of prednisone should be given when necessary

In spite of therapeutical improvements in recent years acute myelogenous leukemia (AML) still carries a poor prognosis Left untreated death usually occurs within months However there exist atypical patients who may live with their disease for many months and even years Rheingold et al (5) described 3 patients with AML who followed a slowly progressive course These authors proposed the term smouldering acute leukemia which has become widely accepted Comments on the criteria and clinical features of this disorder have been surprisingly few considering Rheingold s bold conclusion (4) the best treatment may be no specific treatment at all

In this paper we present our experience with this variant of AML during the last 10 years and propose criteria for the diagnosis

CRITERIA FOR DIAGNOSIS

In the period 1966–76 195 patients with AML were admitted to Medical Department A Hematology Section The material included patients with classical AML and acute myelomonocytic hypergranular promyelocytic and erythroleukemia Cytotoxic treatment was practically always postponed in patients who at the time of diagnosis had less than 30% myeloblasts plus promyelocytes in the bone marrow Provided a second examination in our department after a minimum of 6 months revealed that the patient had not entered a fulminant stage of the disease *the acute leukemia was classified as smouldering*

REPRESENTATIVE CASE REPORT

A 48-year-old man was found to be anemic at a routine examination Other peripheral blood values were within *normal limits and physical examination revealed nothing* abnormal The bone marrow was hypercellular with 10% promyelocytes and 15% blast cells He was observed without treatment and remained free from symptoms About a year later he developed pericarditis and heart failure and was given digoxin with prompt effect He remained slightly short of breath during physical exertion His appetite was good and there was no weight loss He had no bleeding tendency no infections and did not need transfusions until 2 years after the diagnosis was made At that time his Hb was 8 g/100 ml WBC 6000 × 10^6/l and platelets 126000 × 10^6/l Promyelocytes plus blast cells represented 30% of the nucleated cells in the bone marrow He felt still quite well 3½ years after the diagnosis had been made had no tendency to infections or bleeding but needed blood transfusions every 3–4 weeks His peripheral WBC was normal he was moderately thrombopenic and 70% of the nucleated cells in the bone marrow were blast cells

Forty five months after the diagnosis was made he was admitted to hospital with symptoms of malaise Physical examination showed normal results His peripheral blood count was 62000 × 10^6 WBC/l 85% of which *were blast cells and he had 21000 × 10^6 platelets/l* The course was increasingly downhill the need for trans

Table I *Patients admitted with AML during 1966–76*

	Definitely or probably fulminant AML	Smouldering AML	Total
No of pats	183	11	194
Males (%)	53 6	72 7	54 9
Age at diagnosis (y)			
Mean	48 8	65 7	49 8
Range	13–92	48–77	13–92

fusions increased and he became subfebrile Chemotherapy was tried but did not induce remission. He died following a cerebral hemorrhage 47 months after the diagnosis was made

RESULTS

Eleven patients met the criteria set up above The initial morphological diagnosis was classical AML in 4 acute myelomonocytic leukemia in 4 and acute erythroleukemia in 3 patients With one ex- tion all patients had been anemic or had a pan penia for months (5 patients) or years (5 pa ts) One patient had pancytopenia for 8 years fore the diagnosis of acute myelomonocytic leukemia was made These 11 patients with smouldering AML were significantly older ($p<0$ 01 Wilcoxon s rank test) than patients with fulminant AML (Table I) and 8 were men At the time of diagnosis all patients were in good physical condition Two had noticed petechia one had moderate splenomegaly and skin infiltrates chiefly made up of mononuclear cells none had lymphadenopathy Two patients needed antibiotic treatment during the initial diagnostic stay in hospital which usually lasted less than 2 weeks

The majority of patients with smouldering AML were anemic on first admission (Table II) and their mean need for transfusion was 3 2 units of red cell concentrates during the initial diagnostic stay Their other peripheral blood values are given in Table II Only two patients had leukocytosis initially while 6 were leukopenic Seven patients were thrombocytopenic 4 had values within the normal range Blast cells were present in the peripheral blood of 4 patients

The bone marrow was normocellular or hypercellular in 9 of the 11 patients and all three main lines were relatively well preserved considering the underlying disorder (Table II) Auer rods were observed in 2 of the 11 patients i e as frequently as in the patient material at large None had ringed sideroblasts in the bone marrow

The clinical course was slowly downhill The patients lived a reasonably good life The majority stayed in their jobs or continued their activities Admission to hospital was nearly always due to transfusions Long asymptomatic periods were the rule rather than the exception Infections were relatively rare and marrow failure did not occur as a rule until shortly before death Therapy was limited to red cell concentrates and a small dose of prednisone supplemented with symptomatic treatment in 9 patients One patient presented in the case report received specific chemotherapy during the terminal blast crisis Another patient has taken 7 5 mg prednisone and 75 mg cyclophosphamide daily for the last 23 months and is alive and well He presented 28 months ago with marrow and blood findings typical for smouldering acute myelomonocytic leukemia, slight splenomegaly and two skin infiltrates chiefly made up of mononuclear cells After 2 months on this regimen the infiltrates disappeared completely although the bone marrow findings remained unchanged

In the terminal stage the symptoms and signs typical of fulminant AML appeared The platelet count decreased sharply blast cells and promyelocytes accounted for more than half of the nucleated cells in the average patient and leukocytosis was the rule In the 2 patients still alive hematological

Table II *Smouldering AML findings in peripheral blood and bone marrow at the time of diagnosis*

	Mean	Range
Peripheral blood		
Hb (g/100 ml)	9.3	7 2–13 3
Leukocytes (10^9/l)	6 0	0 9–17 7
Platelets (10^9/l)	118	10–350
Blast cells	Present in 4 out of 12 pats.	
Bone marrow		
Cellularity judged from bone marrow biopsy	Normal or increased in 9 decreased in 2 pats	
Megakaryocytes	Normal or increased in 7 decreased in 4 pats	
Erythropoiesis (%)	27	1–60
Blast cells + promyelocytes (%)	19	13–35

Table III *Smouldering AML clinical course*

		N	Median survival after diagnosis (mo)	Anti leukemic therapy (no of pats)
Dead		9	30 (9–48)	1
Cause of death				
Hemorrhage	3			
Septicemia	4			
Unknown	2			
Alive		2	>14 >28	1
Total		11	29	2

75 mg cyclophosphamide and 7 5 mg prednisone daily for 23 months

parameters do not differ appreciably from the values obtained at the time of diagnosis Survival from the time of diagnosis is given in Table III together with the causes of death

DISCUSSION

The diagnosis of a smouldering phase of acute leukemia cannot be based on a single examination A definite but not overwhelming increase in promyelocytes and blast cells in the bone marrow raises the suspicion but observation is necessary to confirm the diagnosis We demanded that the patients remained in reasonable balance with their disease for at least 6 months which is approximately twice the median survival of patients not given specific antileukemic therapy or given therapy but not obtaining remission (2) For patients achieving complete remission on our intermittent combination therapy the median survival is approximately 16 months from start of treatment (2)

Smouldering AML represents a benign course rather than a specific type of AML The most common morphological subgroups of AML were all represented in our patient material The cell density of the bone marrow was variable and anemia or pancytopenia was or became a problem in all of these patients

The obvious question becomes do all patients with AML have an insidious onset which is diagnosed in only a few? We do not think so but cannot prove it To us it appears more important to consider the therapeutical consequences of an early diagnosis of a smouldering stage Our experience confirms that of Rheingold (4) that these patients should be left alone and be given supportive treatment (transfusions antibiotics and a small dose of prednisone) only when needed Aggressive chemotherapy should be considered only during an accelerated phase of the disease and probably not be given to patients above 60 years of age (2) These were the main reasons why only one out of 9 patients who died with smouldering leukemia was treated with antileukemic therapy We do not share the opinion of Beard et al (1) that infective or hemorrhagic complications are indications for chemotherapy

Two points need to be stressed Firstly the incidence of smouldering among our patients with AML (6%) is a minimum number as several potential smoulderers were excluded because they were admitted only once The true incidence is probably appreciably higher because our department receives a selected group of patients with AML For example older patients who are not candidates for intensive chemotherapy are rarely referred from other hospitals It has been suggested that as many as 10–15% of patients with acute leukemia may fall into the smouldering category (6) Secondly smouldering should be distinguished from preleukemia (3) The latter is a marrow dysfunction preceding the onset of an acute leukemia and the term can therefore only be used in retrospect (4) In smouldering AML the bone marrow is diagnostic of AML by usual criteria when first seen

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Reversible Renal Failure Caused by Hypercalcemia

A Retrospective Study

Lars Eric Lins

From the Department of Medicine Renal Unit Karolinska Hospital Stockholm Sweden

ABSTRACT The influence of hypercalcemia on renal function was studied retrospectively in 13 patients suffering from primary hyperparathyroidism, sarcoidosis, vitamin D intoxication, malignant lymphoma or chronic lymphatic leucemia Different kinds of treatment, depending upon the primary disease, often induced a rapid fall in the serum calcium concentration The serum creatinine concentration always fell simultaneously The serum phosphate concentration fell in all but two patients Changes in serum calcium and serum creatinine correlated significantly ($p<0\ 001$) as did changes in serum calcium and serum phosphate concentrations ($p<0\ 05$) Serum calcium/serum creatinine and serum calcium/serum phosphate ratios were significantly higher in patients with primary hyperparathyroidism than in patients with hypercalcemia of non hyperparathyroid origin ($p<0\ 01$ $p<0\ 001$) This suggests a different effect of calcium on the glomerular filtration rate in hyperparathyroid and non hyperparathyroid patients, the latter group being more sensitive to the influence of hypercalcemia Possible explanations for this difference such as a protective effect of PTH on the glomerular filtration are discussed

Hypercalcemia may affect renal function leading to impaired concentrating ability and reduced glomerular filtration with or without demonstrable nephrocalcinosis Renal failure has been described earlier in patients with hypercalcemia secondary to various diseases e g hyperparathyroidism (8) sarcoidosis (16) neoplasms (18) vitamin D intoxication (1 3) vitamin A intoxication (29) immobilization (14) hyperthyroidism (11) and milk alkali syndrome (5) Various degrees of influence on renal function have been reported Treatment with saline phosphate corticosteroids calcitonin or parathyroidectomy has usually normalized serum calcium levels but not always the renal function In some patients function has been fully restored in others renal failure has persisted and some have died of uremia Most authors report impaired tubular function e g impaired concentrating ability Less is known about the hypercalcemic effects on glomerular filtration in man (8 30)

Clinical experience of two patients with transient hypercalcemia and rapidly reversible renal failure inspired us to study the records of 13 patients with hypercalcemia and reversible renal failure The present paper concerns the connection between serum calcium concentration and renal function during treatment of hypercalcemia

PATIENTS

Thirteen patients with hypercalcemia and renal failure were studied retrospectively Age sex diagnoses and treatment are listed in Table I The mean age was 54 years

Of the patients with primary hyperparathyroidism four had chief cell adenomas and one chief cell hyperplasia In all patients with sarcoidosis a Kveim test using the membrane fraction of Kveim suspension (25) gave a positive reaction In two patients one with disseminated sarcoidosis described in detail in another paper (24) mediastinoscopy showed a macroscopic picture typical of sarcoidosis The diagnosis was verified by biopsy from the hilar glands Another patient had enlarged submandibular glands and a biopsy from these showed granulomas The diagnosis of vitamin D intoxication in three patients was based on the case histories and the fact that the serum calcium concentration normalized when vitamin D medication was withdrawn or reduced The diagnoses of malignant lymphoma and chronic lymphatic leucemia in two patients were based on clinical findings and bone marrow examination

Table I *Age sex diagnoses and treatment of 13 patients with hypercalcemia and reversible renal failure*

Pat no	Sex	Age (y)	Diagnosis	Treatment
1	♂	10	Primary hyperparathyroidism	Calcitonin parathyroidectomy
2	♀	65	Primary hyperparathyroidism	Calcitonin parathyroidectomy
3	♂	67	Primary hyperparathyroidism	Calcitonin parathyroidectomy
4	♀	67	Primary hyperparathyroidism	EDTA parathyroidectomy
5	♀	69	Primary hyperparathyroidism	Calcitonin parathyroidectomy
6	♂	29	Sarcoidosis	Corticosteroids splenectomy
7	♂	50	Sarcoidosis	Calcitonin corticosteroids
8	♀	70	Sarcoidosis	Corticosteroids
9	♀	39	Vitamin D intoxication	Withdrawal of vitamin D
10	♀	53	Vitamin D intoxication	Withdrawal of vitamin D
11	♀	62	Vitamin D intoxication	Reduction of vitamin D
12	♂	56	Malignant lymphoma	Calcitonin
13	♀	67	Chronic lymphatic leucemia	Calcitonin

METHODS

Total serum calcium concentration (normal range 2 20–2 60 mmol/l) was measured by atomic absorption spectrophotometry (Perkin Elmer 403) Serum creatinine concentration (normal range 60–120 µmol/l) was determined according to Taussky (27) Serum phosphate concentration (normal range 0 8–1 5 mmol/l) was analyzed by the method of Briggs (4) Serum sodium concentration (normal range 135–145 mmol/l) and serum potassium con entration (normal range 3 4–5 1 mmol/l) were determined the use of an autoanalyzer All chemical analyses re performed as routine tests at the Department of inical Chemistry

Student s *t* test was used in the statistical analyses

RESULTS

Concentrations of serum calcium creatinine phosphate sodium and potassium before and after treatment are shown in Table II Irrespective of the kind of treatment the serum calcium concentration often fell rapidly during treatment the mean concentration was 3 57 mmol/l before treatment and 2 35 mmol/l after treatment Normocalcemia was established in all but one patient (no 12) after 2–75 days (mean 15) of treatment All patients showed a *simultaneous fall in the serum creatinine concentra*tion the mean value before treatment was 224 µmol/l and after treatment 146 µmol/l The serum phosphate concentrations before and after treatment were 1 35 and 1 08 mmol/l respectively Serum phosphate concentration fell in all but two patients (nos 2 and 13) in whom a slight increase was found at the end of the treatment period Serum sodium and potassium concentrations did not change during treatment of hypercalcemia

Fig 1 presents the individual changes in serum calcium and creatinine concentrations during treatment Statistical analysis shows a significant correlation between changes in serum calcium and serum creatinine concentrations ($p<0 001$) The correlation between changes in serum calcium and phosphate concentrations shown in Fig 2 is also significant ($p<0 05$) In all patients except nos 2 and 13 the serum phosphate concentration ran parallel to the serum calcium concentration

Table III gives the individual ratios between serum calcium and serum creatinine concentrations and between serum calcium and serum phosphate before and after treatment *In Table IV the patients* are divided into a hyperparathyroid and a non hyperparathyroid group The serum calcium/serum

Table II *Concentrations in serum of calcium creatinine and phosphate before (B) and after (A) treatment*

Pat no	S-Ca (mmol/l)		S-creatinine (µmol/l)		S-P (mmol/l)	
	B	A	B	A	B	A
1	3 55	2 35	106	80	0 74	0 52
2	3 35	2 40	80	62	0 61	0 97
3	4 00	2 28	230	203	0 97	0 65
4	5 33	2 45	248	88	1 03	0 48
5	3 69	2 10	115	80	0 81	0 78
6	3 25	2 56	150	97	0 94	0 84
7	3 38	2 24	270	168	1 23	0 58
8	3 13	2 48	318	239	1 52	0 81
9	3 10	2 40	301	194	1 16	0 74
10	3 60	2 20	390	310	3 50	3 10
11	2 85	2 29	124	88	1 45	1 29
12	3 90	2 80	283	106	1 49	0 97
13	3 28	2 04	301	186	2 07	2 33
Mean	3 57	2 35	224	146	1 35	1 08

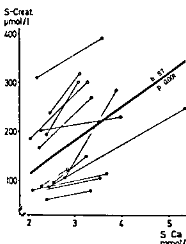


Fig 1 Serum calcium and serum creatinine before (O) and after (●) treatment A regression line is calculated for each subject Mean regression line is expressed by the equation $y=-18\ 4+67\ 1x$ (***)

Table III *Serum calcium/serum creatinine and serum calcium/serum phosphate ratios before (B) and after (A) treatment*

Pat no	S-Ca (mmol/l)/ S-creatinine (µmol/l) B	S-Ca (mmol/l)/ S-creatinine (µmol/l) A	S-Ca (mmol/l)/ S P (mmol/l) B	S-Ca (mmol/l)/ S P (mmol/l) A
1	0 033	0 029	4 80	4.52
2	0 042	0 038	5 82	2 47
3	0 017	0 011	4 12	3 50
4	0 032	0 026	4 56	2 69
5	0 022	0 028	5 17	5 10
6	0 010	0 010	2 06	3 06
7	0 022	0 026	3 45	3 05
8	0 013	0 013	2 75	4 03
9	0 023	0 026	1 97	1 77
10	0 010	0 012	2 67	3 24
11	0 009	0 007	1 03	0 70
12	0 010	0 011	1 58	0 88
13	0 014	0 026	2 61	2 89
Mean	0 020	0 020	3 28	2 92

creatinine and serum calcium/serum phosphate ratios differed significantly between the groups be fore treatment ($p<0\ 01$ $p<0\ 001$) After treatment these ratios did not differ The differences between hyperparathyroid and non hyperparathyroid pa tients suggest that a higher serum calcium level is needed in the former to affect the renal function to the same extent The rapid fall in serum creatinine and phosphate running parallel to the fall in serum calcium is illustrated in Figs 3 and 4

Fig 3 shows the changes in serum calcium and serum creatinine in a patient with sarcoidosis (no 7) He was treated initially with calcitonin and a steroid dexamethasone (Decadron) resulting in a rapid fall in serum calcium and creatinine After three days the calcitonin infusion was discontinued The steroid dose was reduced almost one year later but because of a new hypercalcemic period the higher dose was reintroduced The low grade hypercalcemia seen on this occasion likewise had a reversible effect on the glomerular filtration

The connection between serum calcium and renal function is also clearly shown (Fig 4) in patient 13 with chronic lymphatic leucemia During treatment with anabolic steroids (Anasteron®) cytotoxic agents (Leukeran®) and corticosteroids (pred nisolone) she developed a hypercalcemic crisis with deterioration of glomerular filtration With drawal of the anabolic steroids was followed by a rapid fall in serum calcium and a parallel fall in serum creatinine and phosphate When Anasteron® was reintroduced two weeks later another period of hypercalcemia appeared This time she was treated with high doses of prednisolone and during one day with calcitonin Serum calcium normalized within 5 days The decrease in serum calcium paral lelled a decrease in serum creatinine On both occa sions she developed pronounced and rapidly re versible hyperphosphatemia

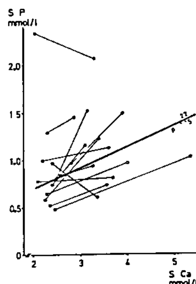


Fig 2 Serum calcium and serum phosphate before (O) and after (●) treatment A regression line is calculated for each subject Mean regression line is expressed by the equation $y=0\ 27+0\ 22x$ ( )

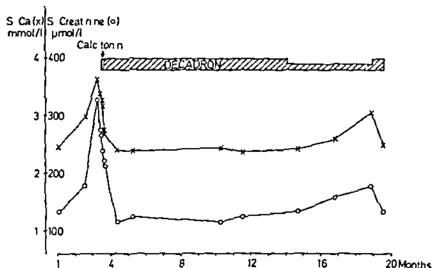


Fig 3 Serum calcium and serum creatinine during treatment of hypercalcemia in patient 7 with sarcoidosis He was treated with Decadron (0 5–1 0 mg i d) and calcitonin (45–135 MRC units i d for three days)

DISCUSSION

Calcium is known to produce tightening of biologic membranes (20) and to inhibit key enzymes of intermediary metabolism such as phosphofructokinase pyruvic kinase and pyruvic carboxylase (6) Calcium also inhibits the Na K ATPase of cell membranes (12) The influence of high calcium concentrations on renal cells might therefore interfere with renal function and even cause cellular death The early destructive lesions of hypercalcemia are found in the ascending limbs of Henle s loop and in the collecting ducts In prolonged and severe hypercalcemia the proximal and distal convoluted tubules also become involved (9) The most common physiological disturbance in calcium nephropathy is impaired concentrating ability (2 30) This has been explained by a defect in the sodium transport in the loop of Henle (10 13 21) by a change in the permeability of the collecting ducts (21) or by intratubular stasis caused by calcium deposits (7) In several cases of calcium nephropathy in man a depression of glomerular filtration rate has been reported In some cases the depression was reversible in others the hypercalcemia caused chronic renal failure This effect of hypercalcemia is poorly studied but some clinical findings are available

In 1949 Burnett et al (5) reported on 6 patients with peptic ulcers treated with excessive intake of milk and alkali for many years These patients all had a marked renal insufficiency with azotemia On a low intake of milk and alkali serum calcium became normal and so did the non protein nitrogen In 1951 Adams (1) described a case of vitamin D induced hypercalcemia with reversible renal failure The normalization of serum calcium and urea nitrogen paralleled one another

Lovice and Connor (18) determined creatinine clearances in 20 patients with hypercalcemia of various origin before and after correction of the hypercalcemia Prior to correction 6 out of 15 patients with hyperparathyroidism had reduced

Table IV *Serum calcium/serum creatinine and serum calcium/serum phosphate ratios before (B) and after (A) treatment (mean ±S D) Comparison between patients with primary hyperparathyroidism and non hyperparathyroid disease*

	S Ca/S creatinine		S-Ca/S P	
	B	A	B	A
Primary hyperparathyroidism (n=5)	0 029±0 010	0 0?6±0 010	4 89±0 64	3 66±1 14
Non hyperparathyroid disease (n=8)	0 014±0 006	0 016±0 008	2 27±0 76	2 45±1 20
p value	<0 01	>0 05	<0 001	>0 05

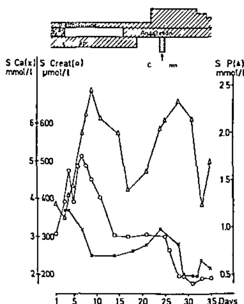


Fig 4 Serum calcium serum creatinine and serum phosphate in patient 13 with chronic lymphatic leucemia She was treated with prednisolone (10–40 mg i d) Anasteron® (100 mg i d) Leukeran® (4 mg i d) and calcitonin (135 MRC units)

creatinine clearance After treatment clearance improved in three remained unchanged in one and was further reduced in two of these patients In patients with an initial normal creatinine clearance value this remained unchanged In patients with hypercalcemia of non hyperparathyroid origin a significantly reduced creatinine clearance was found before treatment Improvement in creatinine clearance was uniform with a mean rise of 20 ml/min following correction of hypercalcemia

Several authors (14 15 19 29) have reported an improvement in glomerular filtration rate after normalization of serum calcium in hypercalcemic patients independent of the origin of the hypercalcemia Others (23 26 28) have not been able to show this In the present study all patients showed an improvement in glomerular filtration rate following correction of hypercalcemia All patients but two displayed a fall in both serum creatinine and serum phosphate In contrast to the results reported by Lovice and Connor (18) the improvement in glomerular filtration rate was seen in patients with hyperparathyroidism as well as in those with hypercalcemia of non hyperparathyroid origin

The mechanism underlying the decrease in glomerular filtration rate in hypercalcemia is not clear The most accepted hypothesis is that the reduced filtration rate is caused by blockage of tubules by calcium deposits This hypothesis is supported by studies of Carone et al (7) who found a close correlation between the degree of reduction in glomerular filtration rate and the extensiveness of intratubular obstruction in dogs Bank and Aynedjian (2) reported similar findings in the rat Although they did not conclude that blockage of tubules was the sole cause of the reduced glomerular filtration rate they considered that it played a prominent role

The rapid improvement in glomerular filtration rate following correction of hypercalcemia in our patients suggests an alternative possibility i e a reversible reduction of glomerular filtration rate due to a direct effect of calcium on the glomerulus This could also explain the close relationship between serum calcium and serum creatinine seen in a patient with post transplant hypercalcemia (17)

Even if all patients in the study irrespective of the origin of the hypercalcemia had a reversible reduction of glomerular filtration rate they differed regarding serum calcium/serum creatinine and serum calcium/serum phosphate ratios (Table IV) Before treatment these ratios were significantly higher in the hyperparathyroid than in the non hyperparathyroid patients After treatment they remained higher in the hyperparathyroid group but the difference was not statistically significant

This suggests a different effect of calcium on the glomerular filtration rate in hyperparathyroid and non hyperparathyroid patients the latter being more sensitive to the influence of hypercalcemia There are several possible explanations for this Since information about the duration of hypercalcemia was not available a difference in this duration as an explanation of the different effect on glomerular filtration rate cannot be ruled out It is also possible that the difference could be explained by different degrees of ionized calcium and/or different levels of PTH in the hyperparathyroid and the non hyperparathyroid patients

In hyperparathyroid patients in contrast to non hyperparathyroid patients hypercalcemia is due to an increased tubular reabsorption of calcium as a consequence of high PTH levels In patients with hypercalcemia of non hyperparathyroid origin PTH levels are known to be low The relative re

sistance to high calcium concentrations, high serum calcium/serum creatinine ratios seen in hyperparathyroidism (17) could be explained by a protective effect of PTH Through a direct effect PTH could increase renal blood flow and glomerular filtration rate (22) Indirectly PTH could improve glomerular filtration rate through an increased tubular reabsorption of calcium reduced hypercalciuria and diminished tubular obstruction

A prospective study concerning the influence of hypercalcemia on glomerular filtration rate and renal blood flow as well as tubular function is in progress Patients with hypercalcemia of various origins, e g primary hyperparathyroidism sarcoidosis vitamin D intoxication and cancer of the parathyroid glands are included in the study

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Serum Inorganic Phosphate and Serum Calcium in Middle-Aged Men

II Relation to Glucose and Insulin Parameters in Glucose Tolerance Tests

Folke Lindgarde and Erik Trell

From the Department of Medicine and Section of Preventive Medicine University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT Fasting serum inorganic phosphate and fasting serum calcium were measured in 752 49 year-old Malmo males, in whom an oral or i v glucose tolerance test, in 189 cases including plasma insulin measurements, was performed as part of an internal medical screening examination The serum inorganic phosphate and calcium levels were analysed statistically in relation to various glucose and insulin response parameters A significant, positive correlation of serum inorganic phosphate with the early insulin response in the i v glucose tolerance tests was found but not with any of the other glucose or insulin response parameters included in the study Serum calcium showed no significant correlations These findings are discussed in relation to the pertinent literature

The factor most strongly and consistently associated with the prevalence of adult onset diabetes mellitus is obesity (24) However insulin and glucose metabolism may likewise be affected by other factors such as age (24) and various hormonal influences There are experimental observations suggesting that inorganic phosphate may be of importance too It is well known that blood inorganic phosphate levels fall after administration of glucose (9) In vitro experiments indicate that insulin mediated glucose transportation over the cell membrane is accompanied by equimolar cellular influx of magnesium and inorganic phosphate (16) Availability of phosphate in the extracellular fluid might thus be one possible factor influencing glucose tolerance

Intracellular calcium plays a fundamental role for hormonal (22) including insulin release (8 17) There are in vitro studies indicating that intracellular calcium pools are influenced by the level of the inorganic phosphate concentration in the medium (3) Energy rich phosphate compounds seem to be directly involved in the insulin secretion process (23) The amount of energy rich phosphate compounds seems to be related to the overall phosphate depots (25)

In order to further evaluate a possible role of phosphorus in the glucose transport/insulin secretion mechanisms and separate it from other influences such as weight age and sex we thought it worthwhile to analyse weight independent partial correlations of serum inorganic phosphate and various glucose and insulin parameters in a sizable age and sex uniform material of glucose tolerance tests A similar analysis of serum calcium was also included in the study

STUDY POPULATION

A medical screening examination further details of which have been given in a previous communication (14) was performed at the Section of Preventive Medicine Department of Internal Medicine Malmo General Hospital between Sept 1974 and Sept 1975 in 1 156 men born in 1926 Serum calcium (S-Ca) was obtained in all individuals A glucose tolerance test (GTT) was also part of the screening procedure in all individuals except those who were overtly diabetic and/or had a fasting glucose level of >120 mg/100 ml Plasma insulin radioimmunoassays were included in the GTT in all markedly overweight individuals (actual/ideal (A/I) weight ≥1 30) and in all individuals of ideal or near ideal weight (A/I weight 0 95–1 05) Serum inorganic phosphate (S-P) was obtained in a random subsample consisting of 752 men with fasting blood glucose of ≤120 mg/100 ml and constituting the present study population An i v or oral GTT with insulin determinations was performed in 47 men with A/I weight ≥1 30 and in 189 with A/I weight 0 95–1 05

Table I *Number and actual/ideal weight (A/I weight) distribution of cases in the various glucose tolerance test (GTT) groups*

Oral GTT without insulin determinations	
A/I weight <0 95 or 1 06–1 29	506
Oral GTT with insulin determination	
A/I weight*	
I	70
II	10
III	18
Sum I–III	98
I v GTT with insulin determinations	
A/I weight*	
I	119
II	0
III	29
Sum I–III	148
Total	752

* I=0 95–1 05 II=1 06–1 29 (1 1–1 25) III≥1 30

Furthermore during the introduction of the method oral GTT with insulin determinations was performed in 10 subjects with A/I weight 1 1–1 25 For the sake of completeness thy are also included in the study (Table I)

METHODS

Analytical methods

All investigations were performed in the morning after an overnight fast The GTTs (*Table I*) were performed in the supine position Fingertip capillary blood samples were used for glucose analyses in the ordinary oral GTT without insulin determinations which was performed in 506 individuals whose A/I weight was <0 95 or between 1 06 and 1 29 In the remaining 246 individuals in whom both blood glucose and plasma insulin were measured an i v catheter for repeated blood samplings was introduced in the left or right antecubital fossa and kept open by saline flushings after the sampling procedures All blood glucose analyses were performed by a standard glucose oxidase method at the Department of Clinical Chemistry Malmo General Hospital Plasma insulin was determined in triplicate with radioimmunoassay technique (11) at the Diagnostic Isotope Laboratory Department of Clinical Chemistry Malmo General Hospital

In the ordinary GTT performed in 506 individuals with A/I weight <0 95 or 1 06–1 29 30 g glucose/m² BSA in a 10% aqueous solution was ingested within 5 min Blood glucose was measured at 0 (immediately before glucose administration) 5 20 40 60 90 and 120 min (in duplicate at 0 and 120 min)

The 189 individuals with A/I weight 0 95–1 05 and the 47 with A/I weight over 1 30 were assigned to a GTT including plasma insulin assays which was performed on a random basis with either oral or i v glucose administration technique In the oral test performed in 70 individuals with A/I weight 0 95–1 05 and 18 individuals with A/I weight ≥1 30 (as well as in 10 individuals with A/I weight 1 1–1 25) 30 g glucose/m² BSA in a 10% aqueous solution was ingested within 5 min Blood glucose was measured at 0 5 20 30 40, 50 60 70 90 and 120 min (in duplicate at 0 and 120 min) and plasma insulin at 0 20 30 40 50 60 70 and 120 min In the i v version performed in 119 cases with A/I weight 0 95–1 05 and in 29 with A/I weight ≥1 30 25 g glucose/m² BSA in a 40% sterile aqueous solution was injected at a constant rate during 3 min Blood glucose was measured at 0 5 20 30 40 50 60 and 90 min (in duplicate at 0 and 90 min) and plasma insulin at 0 5 20 40 and 60 min

Details of weight and height measurements A/I weight calculations according to the formula actual (measured) weight/ideal weight for body length and S-P S-Ca and other laboratory analyses have been given previously (14) At the time of the study the SI metric system was not yet in use Therefore the values of S-Ca are expressed in mEq/l and S-P and blood glucose in mg/100 ml Individuals with fasting blood glucose above 120 mg/100 ml were not included in the study The plasma insulin values were expressed in mIU/ml Normal fasting (time 0) reference values were 5–25 mIU/ml

Statistical methods

The *k* values of i v GTTs were calculated by a computerized method transforming the blood glucose values at 20 30 40 50 and 60 min into a semilogarithmic scale and expressing the correlation coefficient (*r*) and the slope (*k* value according to the standard formula) of the method of least square linear regression analysis of these points *r* values between 0 96 and 1 00 were considered satisfactory

Mean values standard deviation and standard error were calculated according to routine computerized or semicomputerized statistical methods as were the method of least square linear regression analyses and determinations of correlation coefficients In calculating the significance levels of differences between groups Student's *t* test was used Calculation of partial correlation coefficients was made according to standard computerized multivariance analysis methods

RESULTS

Table II gives results of weight independent partial correlation analyses of S P and S Ca with respect to various glucose tolerance parameters No significant correlations were found

Table III shows the weight independent partial correlations of S P and S Ca with various insulin parameters Apart from 10 individuals with A/I weight 1 1–1 25 in the oral version the 246 GTTs during which insulin levels were determined represent weight polarized subgroups of the study population (all cases with A/I weight 0 95–1 05 $n=189$ and all cases with A/I weight ≥1 30 $n=47$) There was no significant correlation between S P or S-Ca and the fasting insulin level The 5 min insulin level

Table II *Mean values (m) standard deviation (S D) and partial correlations of S P and S Ca with (A) blood glucose in all GTTs (B) glucose at 120 min in all oral GTTs and (C) k values in all i v GTTs*
r=Correlation coefficient p=significance level N S =no significance

		Glucose value		Partial correlations	
	n	m	S D	S P	S Ca
A Glucose 0 min (mg/100 ml)	752	76 45	10 02	None	r=0 051 p 0 161 N S
B Glucose 120 min (mg/100 ml)	604	92 80	26 77	r=−0 073 p= 0 074 N S	r=0 039 p=0 331 N S
C k value	148	1 71	0 69	r= 0 114 p= 0 172 N S	r=0 099 p=0 235 N S

in the i v GTTs however exhibited a significant correlation (p=0 025) with S P This positive correlation (p=0 05) also existed between the Δ insulin 5–0 min index and S P S Ca showed no significant associations with any of the i v GTT insulin parameters

In the oral GTTs we analysed the insulin values at 60 and 120 min and found no significant correlations with the S P or S Ca values

In Table IV the weight independent correlation between S P and the 5 min insulin response in i v GTTs in our study population is further illustrated in weight matched S P low and S P high subgroups All i v GTTs in individuals with A/I weight 0 95–1 05 and S P≤2 4 were compared with all individuals with A/I weight 0 95–1 05 and S-P≥3 2 The relative weight was identical in both subgroups and there were no significant differences between the fasting glucose levels k values or insulin levels at 60 min The fasting insulin level was slightly higher in the S P high than in the S P low subgroup but the difference was not significant However the insulin value at 5 min was significantly higher in the S P high than in the S P low subgroup as was the absolute value of the 5 min insulin increase as expressed in the Δ insulin 5–0

Table III *Mean values (m) standard deviation (S D) and weight independent partial correlations of S P and S Ca with (A) plasma insulin at 0 min in all i v and oral GTTs with insulin determinations (B) insulin at 5 min at 5 min minus at 0 min (Δ5–0 min) and at 60 min in all i v GTTs and (C) insulin at 60 min and at 120 min in all oral GTTs with insulin determinations*
I II III as in Table I r p and N S as in Table II

	n				Insulin value (mIU/ml)		Partial correlations	
Insulin	I	II	III	Sum	m	S D	S-P	S Ca
A								
0 min	189	10	47	246	14 625	11 157	r= 0 045 p= 0 483 N S	r−0 080 p=0 436 N S
B								
5 min	119	–	29	148	87 700	49 243	r= 0 187 p= 0 025	r 0 034 p=0 686 N S
Δ 5–0 min	119	–	29	148	73 076	45 884	r= 0 165 p= 0 050	r=0 034 p−0 686 N S
60 min	119	–	29	148	49 121	38 950	r= 0 164 p= 0 058	r=0 086 p=0 342 N S
C								
60 min	70	10	18	98	88 929	48 069	r= 0 039 p= 0 707 N S	r=0 059 p=0 373 N S
1?0 min	70	10	18	98	48 765	42 941	r=−0 052 p= 0 6?0 N S	r=0 093 p=0 370 N S

Table IV *Mean values (m) and standard deviation (S D) of A/I weight blood glucose at 0 min k value plasma insulin at 0 and 5 min Δinsulin 5–0 min and insulin at 60 min and standard error (S E) of insulin at 0 5 and 60 min in S P low and S P high subgroups of 119 subjects with A/I weight 0 95–1 05*

	Subgroup S-P≤2 4 (n=23)			Subgroup S-P≥3 2 (n=27)			Significance of the difference (Student s t test)
	m	S D	S E	m	S D	S E	
A/I weight	1 004	0 034		1 004	0 028		N S
Glucose 0 min (mg/100 ml)	80 74	16 17		77 11	11 09		t=0 966 N S
k value	1 70	0 84		2 00	0 70		t=1 403 N S
Insulin (mUI/ml)							
0 min	10 64	4 46	0 95	13 89	9 78	1 88	t=1 44 N S
5 min	69 17	42 46	8 85	98 07	43 30	8 33	t=2 37 p<0 025
Δ 5–0	58 41	43 02		84 19	39 07		t=2 195 p<0 05
60 min	34 05	16 16	3 37	35 64	18 10	3 62	t=0 31 N S

min value These findings regarding the fasting insulin levels and the insulin levels at 5 and 60 min in the weight matched S P low and S P high i v GTT subgroups are summarized in Fig 1

DISCUSSION

Our main concern here is a discussion of the related to the phosphate and calcium indi when the weight influence is eliminated by partial correlation analysis and by comparison of weight matched S P high and S P low subgroups of the study population The only positive weight independent partial correlations of S P were the 5 min insulin value and the Δ insulin 5–0 min value in the i v GTTs while there was no correlation with the fasting insulin value The same findings were made in the weight matched S P low and S P high subgroups of the study population

The remaining insulin parameters and all glucose parameters included in the statistical analyses lacked significant correlations with the S P values S Ca did not correlate with any of the insulin and glucose indices studied

In experimentally induced long lasting hypophosphatemia in dogs caused by protracted dietary phosphorus deprivation without caloric reduction a mildly impaired glucose tolerance can be provoked (10) This finding agrees with the observation of Maina et al (16) that the transfer of glucose over the cell membrane requires equivalent amounts of phosphate ions However the absence in our study of a correlation between the S P level and the glucose parameters may indicate that within the normal range of S P, to which our study population is confined the concentration of phosphate ions in the extracellullar fluid is sufficient to preclude any appreciable disturbance of the glucose transport over the cell membranes

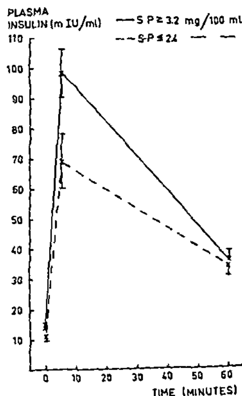


Fig 1 Mean values and standard error of plasma insulin levels at 0 5 and 60 min in S-P low (all cases with S-P≤2 4 mg/100 ml n=23) and S-P high (all cases with S-P≥3 2 mg/100 ml n=27) subgroups of normal weight (A/I weight 0 95–1 05 n=119) i v GTT subjects

Several workers have pointed out that the insulin response to glucose infusion is biphasic (5 10) Initially there is a rapid rise and then a fall in insulin release which has been labeled the acute insulin response The 5 min value obtained 2 min after the end of the 3 min glucose administration that has been used in this study seems to be an adequate representative of the early insulin response (2) After 8–10 min steadily increasing insulin concentrations have been observed and this has been labeled the chronic insulin response

A diminished early insulin response has been observed in individuals with decreased glucose tolerance in manifest or chemical diabetes and in diabetics offspring with normal glucose tolerance (2 7 12) Therefore our finding of a positive correlation between S-P and the early insulin release capacity without any relation to the glucose tolerance seems to be of interest

In the above mentioned dog experiments (10) insulin release in GTTs was increased during phosphate deprivation However the glucose was given i v very rapidly (in 30 sec) and no biphasic insulin release could be observed For several reasons therefore this experiment would seem to represent a different physiological situation than in our study in which there were no apparent phosphate or other dietary deficiencies and no impairment of glucose tolerance

It is well known that acromegalics have high levels of S-P When growth hormone is injected to homo reabsorption of phosphate in renal tubuli increases (4) However an elevation of the plasma phosphate level does not occur until after about four days (4)

Acromegalic patients also seem to have an increased amount *of insulin in the acute release pool* (19) Studies in normal human subjects have shown that after five days pretreatment with growth hormone there is an increase in the maximum insulin response to oral glucose and i v infusion of arginine but no noticeable effect on the glucose tolearance (21) In vitro data support an effect of growth hormone on the β-cell Isolated islets from hypophysectomized rats have a significantly reduced insulin content and a lower insulin output than controls correctible upon three days treatment with bovine growth hormone (18) Similarly in ateliotic dwarfs with low levels of growth hormone there is an insulin deficiency which is correctable by growth hormone administration (21)

However the acute effect of infusing growth hormone in human subjects seems to be inhibition of the insulin secretion by doses chosen to represent the physiological range (1)

It seems reasonable to conclude that the time factor is of importance for both the plasma phosphate and the insulin secretion effects of growth hormone which may suggest an interrelationship We think it would be of interest to extend the study of the phosphate–insulin correlations in our series also to the fasting growth hormone concentration values

The mechanisms involved in the transport of inorganic phosphate over parenchymatous cell membranes are incompletely known In vitro experiments indicate that the level of extracellullar inorganic phosphate influences the amount of intracellular phosphorus (3) During glucose induced early insulin secretion there is an efflux of inorganic phosphate from the *β-cells (6) However the implications* of this finding on the insulin secretion are unclear since the phosphate efflux seems to persist also when the insulin secretion is inhibited by exclusion of Ca^{++} or addition of Ni^{++} to the in vitro system (7)

It seems highly likely that the early insulin secretion is occurring by exocytosis in which energy-rich phosphate compounds are involved In the effluent from perfused rat islands there is a relationship between insulin and adenine nucleotides (25) Experiments with uncoupling agents have shown that the turnover of ATP in mouse islets is extremely rapid (2) Glucose stimulation increases the oxygen consumption of isolated mice islets (12) and therefore presumably their ATP turnover During glucose stimulation of isolated islets there is initially a dramatic fall in ATP levels (19) plausibly re*flecting the expenditure of ATP for energy-depend*ent phosphorylation However there is also a concomitant rapid resynthesis of ATP during the intracellular glucose metabolism (2) Under these circumstances it seems possible that availability of phosphate ions for ATP resynthesis may be an important limiting factor for insulin release capacity It might be conjecturised that high serum inorganic phosphate levels could provide adequate intracellular phosphate stores avoiding a hypothetical shortage of phosphate ions during the initial sponse of the β-cells to glucose This v consistent with the finding in our r higher early insulin response in S-P h als

We found no correlation between the glucose and insulin parameters and the S-Ca values This probably reflects the narrow homeostasis of S Ca It has been postulated that chronic hypercalcemia may represent the primary mechanism responsible for the hyperinsulinemia and carbohydrate intolerance occasionally seen in primary hyperparathyroidism (13 26) Our study did not include any individuals with hyperparathyroidism or hypercalcemia It may be concluded that in the normal range of S Ca no obvious relation can be traced between the level of this ion and glucose transport/insulin secretion indices in GTTs

We have earlier described an inverse correlation between serum phosphate and body weight (14) In the light of the results of the present study it seems to be of interest to consider associations with S P in a forthcoming analysis of the relations between body weight and GTT parameters (15)

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Humoral and Cellular Immunity in Sarcoidosis

N K Veien, F Hardt G Bendixen J Genner
J Ringsted J Wanstrup A Wiik and E Christiansen

From the Department of Dermatology Finsen Institute the Departments of Medicine II and Clinical Chemistry Kommunehospitalet the Departments of Medicine (TA) and Infectious Diseases University Hospital Copenhagen and the University Institute of Pathological Anatomy Odense Denmark

ABSTRACT The Kveim reaction was studied in vivo in 50 patients with sarcoidosis Commonwealth Serum Laboratories Kveim material and a new Danish Kveim material gave 14 and 8 positive reactions respectively, as well as 6 and 8 equivocal reactions Forty six of the patients were also tested in vitro for cell mediated immunity to the Danish Kveim material, using both the leucocyte migration agarose technique and the capillary technique No significant migration inhibition or stimulation were found A tuberculin skin test was performed in 49 of the patients, and in 45 a dinitrochlorobenzene sensitivity titer was determined Both tests revealed a depression of the cell mediated immunity The serum levels of immunoglobulins IgG IgA, IgM IgD, and IgE were determined The serum of each patient was also examined to determine if organ non specific and granulocyte-specific antinuclear factors of IgG class antibodies against native DNA, rheumatoid factor, mitochondrial antibodies antibodies against thyroid cytoplasm and parietal cell antibodies were present IgG levels were above normal in 28 patients, IgE was above normal in 10 patients, 4 of whom were atopics or had an atopic disposition Organ non specific antinuclear factors were present in 17 patients

Sarcoidosis is a multisystem disorder of unknown etiology The fundamental lesion is a non caseating epithelioid cell granuloma Granulomas are formed probably around an almost insoluble antigen—either by a specific immunological mechanism involving specifically sensitized T cells and macrophages (immunological granuloma) or by a non specific reaction (non immunological granuloma) (22)

In some patients with sarcoidosis epithelioid cell granulomas can be induced by intracutaneous injection of sarcoid tissue (Kveim material) which may contain the specific antigen

The presence of antibodies against various microorganisms seems to have no particular significance and previous studies of autoantibodies in patients with sarcoidosis have been inconclusive (17)

The aim of this study was to elucidate the nature of the Kveim reaction by in vivo and in vitro methods to evaluate the cellular immune function in patients with sarcoidosis by in vivo tests and to determine the levels of serum immunoglobulins and various autoantibodies

PATIENTS

The study comprised 50 patients with a clinical picture of sarcoidosis in whom epithelioid cell granulomas consistent with sarcoidosis were found in a biopsy from at least one organ system The patients ranged in age from 17 to 73 years (mean 45) 18 were males and 32 females All patients had been vaccinated with BCG the majority during childhood Twelve patients had only skin involvement one had only hilar adenopathy and in the remainder multiple organ involvement was seen The duration of the disease was less than 2 years in 20 patients and more than 2 years in 30

In order to judge organ involvement clinical radiological ECG and hematological evaluations were carried out

Twenty patients including patients with tuberculosis ulcerative colitis and leg ulcers served as controls for the Kveim tests For the dinitrochlorobenzene (DNCB) testing 45 and for the tuberculin skin test 49 healthy persons served as controls For the in vitro tests 38 persons with no systemic medical disorders served as controls

METHODS

The following test suspensions were used

1) Danish Kveim material (DKM) 1

Table I *Results of histological evaluation of biopsies from sites injected with a Danish prepared Kveim material (DKM) Commonwealth Serum Laboratories Kveim material (CKM) a normal spleen homogenate and a silicium suspension*

	Positive reaction	Equivocal reaction	Negative reaction	Total
DKM	8	8	34	50
CKM	14	6	30	50
Normal spleen homogenate	0	0	50	50
Silicium suspension	1	0	49	50

splenic tissue obtained at autopsy of a patient with sarcoidosis verified histologically and by the clinical picture as well as from the presence of epithelioid cell granulomas in the spleen The splenic tissue was homogenized in a glass homogenizer (Ultra Turrax) and a Potter Elvehjem homogenizer rinsed with phosphate buffered saline (PBS) and centrifuged at 5 000 g for 15 min After rinsing the material was heat inactivated twice (56 for 15 min each time) After centrifugation at 2 000 rpm for 15 min the supernatant—Kveim material—was sterilized by gamma irradiation (1 Mrad) 1 g of splenic tissue was used for preparation of 1 ml Kveim material

2) A suspension of normal splenic tissue prepared from a healthy patient in the same manner as described for the

A suspension of silicium prepared by grinding sand mortar while adding PBS Electron microscopic crystallography was performed on the final suspension to confirm the presence of silicium particles

All patients had 0 1 ml of each of these three suspensions injected intradermally In addition 0 1 ml of Commonwealth Serum Laboratories (CSL)-006-2 Kveim material (CKM) (supplied by Dr T Hurley CSL Melbourne Australia) was injected in the same manner Biopsies from the test sites were coded and a histological evaluation was performed blindly by two pathologists

A positive reaction was defined by the following criteria epithelioid cells which formed granulomas giant cells when present sparse inflammation and absence of birefringent material

The term equivocal reaction denotes the presence of scattered histocytes and epithelioid cells without granuloma formation Lymphocytic inflammation was a constant feature No birefringent material was present

A tuberculin skin test was performed in 49 of the patients starting with 0 1 tuberculin units (TU) purified protein derivative This amount was increased gradually (1 10-100 TU) if the reaction was negative More than 5 mm induration at the 72 hour reading was required for a positive reaction

A DNCB sensitivity titer was determined for 45 of the patients The sensitizing dose was 1 mg in acetone applied with 0.5 mg on each of two filter paper discs with a diameter of 5 cm These discs were placed on the upper back and occluded with tape for 48 hours Four weeks later closed patch tests with DNCB in acetone in concentrations of 0 25–64 μg were applied to another site on the back and read after 48 and 72 hours The lowest DNCB concentration resulting in a 1+ positive patch test was chosen as the DNCB sensitivity index

Immediately preceding in vivo tests 46 of the above mentioned patients were tested in vitro for cell mediated immunity to the DKM The following two tests were used for these studies

1) The leucocyte migration agarose technique as described by Clausen (6) with a few modifications Non-toxic pre incubation concentrations of the Kveim material of 250 and 500 μg protein per ml medium were used in all tests Normal splenic tissue in the same concentrations was used as the control antigen Splenic material or a comparable, antigen free control solution was added to samples of leucocyte suspensions the final cell concentration was 2.1×10^6/ml The cell suspensions were incubated with the splenic material at 37°C for 90 min After incubation the leucocytes were placed in the agarose gel holes Four to six identical migration cultures were prepared from each pre incubation cell suspension After 24 hours the migration areas were measured The plate to-plate and hole to-hole variation between the migration areas of identically treated cultures was less than 7% The migration index (MI) was calculated on the basis of quadruplicates as the ratio between the average migration area of splenic material containing cultures and the average migration area of cultures without splenic material or with control splenic material

2) The leucocyte migration capillary technique was performed as described by Bendixen and Søborg (3) micromodified according to Maini et al (14) for 23 of the patients The highest concentration of sarcoid and normal splenic material was 300 μg protein per ml Quadruplicate cultures were prepared with 100 200 and 300 μg protein per ml Within one set of identical cultures the variation from one migration to another did not exceed 5% Calculation of MI was performed as described for the agarose technique

Serum concentrations of IgG IgA and IgM were quantitated by rocket immunoelectrophoresis (23 24) Serum levels of IgD were also measured by rocket immunoelectrophoresis (12) and IgE levels by a radioimmunosorbent technique (11)

Organ-non-specific and granulocyte specific antinuclear factors (ON ANF and GS ANF) of the IgG class were studied using an indirect immunofluorescence technique (26 27 28) Fluorescein isothiocyanate labelled rabbit IgG specific for human γ-chains (Dakopatts Copenhagen) was used after specificity had been assured by crossed immunoelectrophoresis and direct immunofluorescence tests on monoclonal bone marrow specimens (27 28) All results were read in a Leitz Orthoplan epi illumination fluorescence microscope (26) Both ON ANF and GS-ANF reactions were considered positive if the titres were 16 or more (25)

Antibodies against native DNA were studied by a modified Farr technique (15 29)

Rheumatoid factors were demonstrated by F II latex fixation slide tests (RA test Hyland Brussels) Titres of 32 or more were considered positive

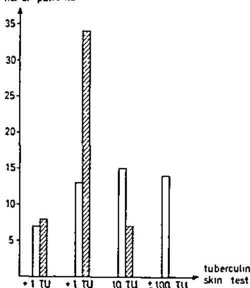


Fig 1 Results of tuberculin skin test of 49 sarcoidosis patients (□) and matched controls (▨)

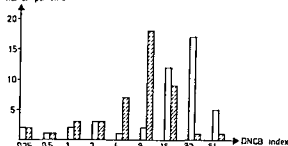


Fig 2 DNCB index for 45 sarcoidosis patients (□) and matched controls (▨)

RESULTS

Table I gives the results of the in vivo testing with the Kveim materials the normal spleen homogenate and the silicium suspension All the positive reactions with the DKM were seen in patients who also had positive reactions to the CKM Five of the 8 equivocal reactions with the DKM were found in patients who had a positive Kveim test using CKM while 5 of the 6 equivocal reactions with the CKM occurred in patients who did not show a positive reaction to DKM One Kveim negative patient did react with granuloma formation when tested with the silicium suspension No positive or equivocal reactions were seen in any of the 20 control patients with any of the 4 suspensions

We did not find significant migration inhibition or stimulation in any of the 46 patients tested in vitro (Table II)

The results of the tuberculin skin tests in 49 of the patients with sarcoidosis and 49 matched controls are shown in Fig 1 The tuberculin sensitivity is seen to be lower in the patient group The DNCB sensitivity for 45 of the patients with sarcoidosis and 45 matched controls is recorded in Fig 2 showing a similar depression of the cell mediated immunity in the patient group

The immunoglobulin levels are recorded in Fig 3 An increased level of IgE was found in 10 patients 4 of whom had clinical manifestations of atopy or a close relative with atopy

IgG ON ANF was present in 17 patients in titres of 1/16–1/128 IgG GS ANF was not found No correlation was obtained between the presence of ANF and the activity or extent of the disease Only one patient with ANF received medication (Levothyroxin 0 4 mg daily) Age and sex had no significant influence on the presence of ANF which was found in 6 of 25 patients under the age of 50 and in 11 of 25 patients over 50 ($p>0\ 1$ χ^2 test) ANF was present in 4 of 18 male patients and in 13 of 32 female patients ($p>0\ 1$ χ^2 test) Antibodies against

Table II *Leucocyte migration inhibition (MI±S D) induced by sarcoid spleen homogenate and normal spleen homogenate in patients with sarcoidosis and in controls using the agarose assay (LMAT) and the capillary tube assay (LMCT)*

MI=migration index S D =standard deviation

	Sarcoid spleen homogenate			Normal spleen homogenate		
LMAT	500 µg/ml	250 µg/ml		500 µg/ml	250 µg/ml	
Patients (n=46)	0 99±0 10	1 00±0 10		0 95±0 10	0 98±0 14	
Controls (n=38)	0 97±0 10	0 95±0 07		0 98±0 10	0 99±0 08	
LMCT	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/
Patients (n=23)	0 96±0 10	0 88±0 10	0 86±0 10	1 00±0 00	1 03±0 10	1 01±
Controls (n=14)	0 96±0 14	–	0 96±0 10	1 00±0 14	–	0 9

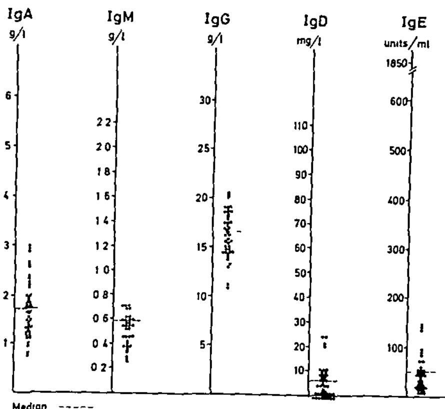


Fig 3 Serum levels of IgA IgM IgG IgD and IgE in 50 patients with sarcoidosis Normal values for IgA <0 54 <3 5 g/l (24) for IgM <0 21<1 44 g/l (24) for IgG <6 8<16 g/l (24) for IgD <150 mg/l (Medicinsk Laboratorium Copenhagen unpublished data) and for IgE <150 U/ml (Kjellman and Johansson unpublished data)

ve DNA (>10% binding) were not found in any e patients

The numbers of patients who had mitochondrial antibodies antibodies against thyroid cytoplasm and parietal cell antibodies in the serum were comparable to those in a normal population (16) The determination of these antibodies was carried out by Statens Seruminstitut

The prevalence of rheumatoid factor was not different from that of a normal population

DISCUSSION

Since Kveim in 1941 introduced the intracutaneous diagnostic test for sarcoidosis there has been much discussion of the test s specificity (10 18) Our results of the in vivo Kveim tests indicate that a positive Kveim test is specifically associated with the injection of a sarcoid tissue suspension The fact that a silicium suspension rarely produces granulomas indicates that the tendency among sarcoid patients to produce granulomas by non immunological stimulation does not give rise to a great number of false positive tests

The ethics of injecting a crude tissue suspension has made in vitro diagnostic tests desirable In 1967 Søborg and Bendixen introduced the leucocyte migration test for the demonstration of cellular immunity This test has been applied using Kveim material as the antigen in patients with sarcoidosis with varying results (2 9 13 19) We have been unable to reproduce our previous positive results possibly due to variations in the potency of the Kveim substances (9) In vivo however there was some activity of the DKM used in the in vitro tests If an immunologically specific lymphokine dependent inflammation is of significance for the development of the sarcoid granuloma one would expect to register this in vitro Our findings suggest that the supposed Kveim antigen is not present in available form for in vitro reactivity with lymphocytes with the techniques used—or that the Kveim reaction is not a T cell dependent reaction

The high serum IgG levels in the patients of this study are in agreement with previous investigations (17) This could be explained by the presence of infections caused by microorganisms such as Epstein Barr virus (21) Mycoplasma (8) and atypical Mycobacteria (5)

Hanngren et al (7) have proposed an interaction

between virus and mycobacterial products in the pathogenesis of sarcoidosis According to this hypothesis a virus (unknown) depresses T cell function and tuberculo-protein enhances immunoglobulin formation Another possible explanation is that a depression of T cell function by a virus decreases the suppressor effect of T cells on B cells (1) This would explain the high IgG levels and the presence of ANF in a large proportion of our patients ANF is also encountered in other chronic inflammatory diseases (20)

Previous investigations of the levels of circulating IgE in patients with sarcoidosis have yielded conflicting results Parallel to our results Bergman et al (4) found significantly higher IgE levels among patients with sarcoidosis than among controls Yagura et al (30) on the other hand noted an IgE deficiency in a group of patients with sarcoidosis These discrepancies could be due to the selection of controls

The prevalence of ANF in our group of patients is higher than that previously reported in patients with sarcoidosis The reasons for this are not clear Only one patient was receiving medication ANF production was not drug induced which may indicate that increased amounts of IgG ON ANF can be produced in untreated cases of sarcoidosis This probably reflects an increased demand for removal of nuclear debris from sarcoid lesions The recent detection of lymphocytotoxic antibodies in about 20% of sarcoidosis patients (Horatius unpublished data) could indicate an increase in the breakdown and removal of lymphocytes which may also stimulate ANF production The results of the DNA binding assay indicate that the ANF specificity is not directed toward native DNA A generalized increase in auto-antibody production did not take place as witnessed by the absence of mitochondrial parietal cell and thyroid antibodies as well as rheumatoid factors

ACKNOWLEDGEMENT

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Bleomycin Treatment in a Case of Polyarteritis Nodosa

Some Immunological Studies

Henric Blomgren J V Rebekka Berg Folke Edsmyr Renee Norberg
and Jerzy Wasserman

From Radiumhemmet Karolinska Hospital the Central Microbiological Laboratory of the Stockholm County Council and the National Bacteriological Laboratory Stockholm Sweden

ABSTRACT A 65 year old man presented himself with a tumorous mass which had developed during a period of about 4 years The lesion was similar to a penile carcinoma although no malignant cells were found in biopsies Accordingly he was treated with local radiotherapy and injections of bleomycin, which resulted in an almost complete regression The patient, however, died in a state of uremia 2½ months after initiation of the treatment Hepatitis B surface antigen (HBsAg) could not be demonstrated whereas antibodies against HBsAg were present in the patient's sera before, during and after treatment A diagnosis of polyarteritis nodosa, localized to the kidneys and genital organs, was revealed by the postmortal histologic examination Cancer cells however, were not observed Before the patient entered treatment his peripheral lymphocytes exhibited weak proliferative responses to phytohemagglutinin (PHA), concanavalin A (ConA) and PPD tuberculin *in vitro* The frequency of lymphocytes binding sheep erythrocytes, E cells (T cells), was also subnormal The Ig levels, C3 and C4 were within normal ranges Within a few weeks after the start of therapy the reactivity of lymphocytes to ConA and PHA increased sharply There was also some increase in the PPD reactivity of the lymphocytes and the frequency of E-cells was normalized The IgA and IgG levels in serum increased One explanation for the rapid progression of polyarteritis nodosa could be that the bleomycin treatment corrected the immune deficiency of the patient and thereby accelerated the vasculitis, which is probably of an immunological nature

Polyarteritis nodosa is a relatively rare condition which is characterized by inflammatory changes in blood vessels especially small and medium arteries The disease may have a chronic course with remissions and exacerbations or an acute transient or fulminant course Depending on its localization the respiratory gastrointestinal renal hematopoietic musculo-cutaneous skeletal central or peripheral nerve systems may be affected

The etiology of the disease has not been definitely established but an immunological mechanism has been suggested (11) Since Gocke et al (12) in 1970 described this condition in association with the presence of hepatitis B surface antigen (HBsAg) several cases have been described where HBsAg and/or the corresponding antibody (anti HBs) has been demonstrated before during or after the onset of vasculitis (9 21) Trepo et al (27) found that 69% of their histologically confirmed cases demonstrated either HBsAg or anti HBs 11% both and 17 7% anti HBs alone Polyarteritis nodosa has also been seen in association with serum sickness drug abuse rheumatoid arthritis serous otitis media and streptococcal infections

Peripheral lymphocytes of patients with polyarteritis nodosa do not exhibit significant proliferative responses to preparations of artery antigens in vitro this is in contrast to lymphocytes from subjects with polymyalgia rheumatica (15) which is a disease frequently associated with vasculitis with or without anti HBs (1) This indicates that immunological responses to exogenous factors rather than autoimmunity may be the cause of polyarteritis nodosa

In this article we present the results of some immunological tests performed on serum speci

and peripheral lymphocytes from a patient with vasculitis most probably polyarteritis nodosa who was treated with bleomycin a relatively new anti cancer agent

MATERIAL AND METHODS

Blood sampling Capillary blood was collected for de terminations of Hb concentration (g/l) number of platelets and leukocytes ($\times 10^9$/l) Differential counts were made on methanol fixed smears after May-Grunwald and Giemsa staining Venous blood drawn into heparinized tubes served as a source of lymphoid cells and venous blood collected in non heparinized tubes served as a source of serum

Separation of serum Blood was stored over night at +4 C After centrifugation the serum was collected and stored at −20°C before being analyzed

Separation of lymphoid cells Lymphoid cells were separated from blood by centrifugation on a layer of Ficoll Isopaque (17) The cells were suspended in Eagle s minimal essential medium supplemented with Earle s salts (MEM)

Frequency of T-cells Lymphoid cells suspended in MEM were depleted of monocytes macrophages by add ing carbonyl iron powder followed by removal by a magnet (3) The remaining preparations of lymphocytes were assayed for frequency of cells capable of binding sheep red blood cells (SRBC or E-cells) using a method described previously (5) E-cells are considered to be ells (17) At least 200 cells of each cell preparation cored

te stimulants Lymphocytes were stimulated the following agents 1) Phytohemagglutinin (PHA) phytohaemagglutinin M Difco Lab Detroit Mich) The contents of commercially available vials of PHA were dissolved in 5 ml of MEM This concentration will be referred to as 100% of PHA 2) Concanavalin A (ConA) (Sigma Chemical Co St Louis Mo) The con centration of ConA used is expressed as μg/ml 3) Purified protein derivative of tuberculin (PPD) (RT 22 Statens Seruminstitut Copenhagen Denmark) The concentra tion of PPD is expressed as μg/ml PHA and ConA are polyclonal phytomitogens which predominantly stimulate T-cells to DNA synthesis whereas PPD is an antigen which mainly stimulates specifically sensitized T-cells

Lymphocyte cultures A previously described micro-culture technique was used (18) Briefly 5×10^4 lympho-cytes were cultured in the wells of microtest plates con taining 0 2 ml of MEM supplemented with 10% of heat in activated human serum penicillin and streptomycin The cultures received PHA ConA or PPD at concentrations indicated in the text Control cultures received no stimu-lants After four days of cultivation at 37°C in a humidified 5% CO_2 air atmosphere the cultures received 1 0 μCi of ^{3}H thymidine (5 Ci/mM) Twenty four hours later the cultures were terminated and incorporated radioactivity expressed as cpm was determined as described elsewhere (18) Mean values of duplicate cultures were calculated on an arithmetic basis Isotope uptakes of control cultures which did not exceed 300 cpm were subtracted from the respective experimental cultures

Experimental design of lymphocyte stimulations There is frequently a high interexperimental variation of the stimulations of lymphocytes obtained from the same healthy donor using various mitogenic agents in vitro This variation is probably due to uncontrolled changes of the culture conditions To circumvent this variability the lymphoid cells of a healthy control were always cultured in parallel with the patient s lymphocytes The isotope uptakes of the patient s cells were related to the value of the control lymphocytes and expressed as per cent

Quantitation of serum immunoglobulins The amounts of IgG IgA and IgM were determined by radial immuno-diffusion (19) The normal value of IgG was 11 18±2 06 of IgA 1 97±0 65 and of IgM 0 74±0 24 g/l IgE was determined by a commercial kit (Pharmacia Uppsala, Sweden) and expressed as U/ml Values of normal sera are stated not to exceed 100 U/ml

Quantitation of serum complement factors Comple-ment factors C_3 and C_4 were determined by radial immunodiffusion in Hyland's and Behringwerke agar plates The assays were standardized against normal hu-man serum Mean levels in healthy controls were 1 40±0 35 g/l for C_3 and 0 26±0 12 g/l for C_4

Tests for presence of autoantibodies Presence of serum autoantibodies was determined by an indirect immuno-fluorescence technique employing FITC-conjugated heterospecific sheep antihuman immunoglobulin (Na tional Bacteriological Laboratory Stockholm Sweden) and cryostat sections of rat kidney rat stomach and hu man thyroid The initial dilution of the patient s serum was 1 10

Tests for Clq-binding capacity and for rheumatoid factor The latex RA agglutination test (Hyland) was used modified according to Svehag and Burger (26) Briefly the patient s serum specimens were diluted serially two-fold in borate buffer the latex reagent was diluted 1 4 in the same buffer made 0 05 M with respect to Na 3 H EDTA (Svehag personal communication) To give clear-cut end points normal guinea pig serum heat treated at 56°C for 30 min was added to the EDTA buffer to a final dilution of 1 100 This addition did not alter the titers the trailing of the reactions however was eliminated The test was set up in duplicate In one type of test (I) the patient s sera were untreated in the other type (II) the sera were heat treated at 56 C for 30 min to inactivate the first part of the first component of complement (Clq) ac cording to Cheng and Persellin (6) The Clq-binding capa-city was measured in test I A titer of 32–128 was consid-ered as normal Test II was used for the demonstration of rheumatoid factor (RF)

Test for antiviral antibodies The patient s sera were screened at a dilution of 1 8 using a complement fixation (CF) test (25) against 15 viral antigens Mycoplasma pneumonia and ornithosis antigens were added (23)

Tests for HBsAg and anti HBs A radioimmunoassay (Ausria II 125 Abbott Laboratories) was employed

Tests for antibodies against bleomycin The patient s sera undiluted and diluted 1 2 were tested for antibodies against bleomycin Bleomycin at various dilutions was used as antigen The reactants were tested in immuno-diffusion (2) immunoelectroosmophoresis (14) and CF test

Table I *Peripheral blood picture of the patient before during and after treatment*

Date of examination	Hb (g/l)	No of platelets ($\times 10^9$/l)	No of leukocytes ($\times 10^9$/l)	Frequency of cells (%)				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Dec 15 1975 (start of treatment)	122	280	9 1	73	2	1	21	3
Jan 1 1976	108	330	7 0	71	12	1	10	6
Jan 19 1976	121	410	9 1	71	7	2	16	4
Feb 5 1976 (after treatment)	112	415	11 7	69	9	3	12	6
Feb 19 1976	105	475	10 2	79 5	7	0 5	8	6

Treatment of the patient The patient received combined treatment with local radiotherapy directed to the entire penis shaft and systemic administration of bleomycin The penis was irradiated from two opposing beams of a conventional X ray machine The physical factors were 190 kV 20 mA focus skin distance 40 cm 0 5 mmCu The patient was irradiated 5 days a week for 5 weeks The total mid-dose was 5800 rads Every second week the patient received an i m injection of 15 mg bleomycin (see below) 2 h before each irradiation (total dose 225 mg) This schedule is currently used at the Radiumhemmet for treatment of penile carcinoma (10)

Bleomycin The drug was supplied by AB H Lundbeck and Co Malmo Sweden According to the classification of Umezawa et al (28) the bleomycin preparation given contained the following peptide antibiotics A_2 55–70% A_2 less than 7% and B_2 25–32%

CASE REPORT

The patient a 65 year old man who had previously been healthy attended the Karolinska Hospital in Nov 1975 for a tumorous mass on the glans of the penis He had noticed an ulcer on the glans in 1972 or 1973 which healed spontaneously leaving an indurated area In July 1975 he had a sensation of itching on the glans and thereafter there developed a local edema At the time of admission the entire glans was transformed into a bluish small nodular tumorous mass with several bleedings

Biopsies taken on three occasions from different parts of the glans showed a histologic picture of chronic inflammation with squamous cell hyperplasia Malignant cells were not observed Physical and chest X ray examination did not reveal any further pathological changes ESR rose from 33 mm in Nov to 73 mm in Dec the same year Wassermann tests were negative as well as examinations for presence of Neisseria gonorrhoeae

After a ten-day course of local antibiotics which failed to improve his condition the patient entered local radiotherapy combined with injections of bleomycin according to a schedule used for treatment of penile cancer since the lesion was macroscopically strikingly similar to a penile carcinoma Treatment which was started on the 15th of Dec 1975 and completed at the end of Jan 1976 resulted in an almost complete regression of the tumorous lesion

In the beginning of March 1976 the patient developed a thrombosis in his left leg which was successfully treated with anticoagulants At that time the patient had for the first time an elevated concentration of creatinine in his serum (2 6 mg/100 ml normal range 0 6–1 4) In the middle of March the creatinine concentration rose above

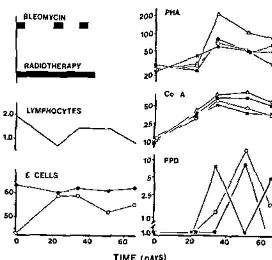


Fig 1 Changes in the peripheral lymphocyte population ($\times 10^9$/l) before during and after treatment with local radiotherapy and injections of bleomycin The periods for these treatments are indicated by shaded areas Frequency of E-cells (T cells) of the patient (O—O) and a *control tested in parallel* (●—●) *DNA synthetic re*sponses of the patient s lymphocytes to mitogenic agents are expressed as per cent of the respective values of a control who was tested in parallel Responsiveness to PHA at a concentration of 3% (×—×) 1% (O—O) 0 3% (●—●) and 0 1% (Δ—Δ) Responsiveness to ConA at a concentration of 14 μg/ml (×—×) 7 μg/ml (O—O) 3 5 μg/ml (●—●) and 1 7 μg/ml (Δ—Δ) Responsiveness to PPD at a concentration of 10 μg/ml (×—×) 1 0 (O—O) and 0 1 μg/ml (●—●)

Table II *Serum levels of immunoglobulins C3 and C4 before during and after treatment (g/l except for IgE which is expressed as U/ml)*

Date of examination	Designation of serum sample	IgM	IgG	IgA	IgE	C3	C4
Dec 15 1975	I	0 95	13 1	1 70	15	1 82	0 16
Jan 1 1976	II	1 05	13 5	1 80	11	2 58	0 18
Jan 19 1976	III	1 05	17 7	1 80	10	2 58	0 20
Feb 5 1976	IV	1 10	19 9	2 50	10	3 00	0 22
Feb 19 1976	V	1 10	19 9	2 25	12	2 52	0 11

15 mg/100 ml and subsequently the patient died in a state of uremia

Macroscopic examination of the organs at autopsy showed several infarct like changes of both kidneys and microscopic examination of the kidneys and genital organs showed strong inflammatory reactions around blood vessels with accumulations of lymphocytes plasma cells eosinophils and neutrophils The media of the walls of several vessels had undergone fibrinoid necrosis The histological picture indicated the diagnosis of polyarteritis nodosa Cancer cells were not observed

IMMUNOLOGICAL RESULTS

Table I and Fig 1 show the peripheral blood picture the reactivity of the patient s lymphocytes to genic stimuli before during and after combined tment with bleomycin and local irradiation Apart from a relatively mild anemia the peripheral blood picture was fairly normal before the patient entered therapy The frequency of E cells however was low (43%) and the response of the lymphocytes to the phytomitogens PHA and ConA were highly suppressed at all concentrations employed and the lymphocytes did not exhibit any detectable reactivity to PPD-tuberculin in vitro Treatment resulted in a rapid increase in the number of platelets and a clearly increased frequency of eosinophils The proportion of E cells was also increased and the reactivity of the lymphocytes to PHA and ConA increased sharply This change was particularly evident at the lowest concentrations of the mitogens employed where 10-fold increases were observed A weak PPD reactivity also appeared at the end of the treatment

Serum Ig levels and concentrations of C3 and C4 were within normal ranges at the start of the treatment There was an increase in the level of IgG after treatment and such a tendency was also noted for IgA and C3 (Table II) Determinations for presence of serum autoantibodies against nuclear factors cytoplasmic thyroid antigen smooth muscle mitochondria and glomerular elements gave negative results in all tests

All serum specimens lacked CF antibodies at dilution 1 8 anti HBs however was present in all serum samples Circulating HBsAg was not detected Antibodies directed against bleomycin were not detected in any of the serum samples

The Clq binding capacity of all serum samples tested was within the normal range RF was present in low titer 2–4

DISCUSSION

Although malignant cells could not be demonstrated in biopsies the patient s lesion was strikingly similar to a penile carcinoma Accordingly he was treated with local irradiation and bleomycin Polyarteritis nodosa localized to the penis has not been described before and was therefore not considered It is not known whether the lesion on the glans was a manifestation of the patient s vascular disease

The onset of the patient s vasculitis and its etiology are unknown The lesion however that brought him to treatment had developed for approximately four years It is of interest that anti HBs was present in the patient s sera since other investigators have detected HBsAg and/or anti HBs in a large proportion of cases with polyarteritis nodosa (21 27) For instance Trepo et al (27) using radioimmunoassay detected HBsAg in 54% of their patients with periarteritis nodosa and anti HBs in 17% while 11% had both HBsAg and anti HBs There is evidence that the development of anti HBs induces remission of the disease (9 27)

We like others (7 21) failed to detect immune complexes in the sera of the patient The method we have employed may however be insensitive

since others have detected HBsAg–anti HBs complexes using other methods (27)

The peripheral lymphoid cells of the patient exhibited weak responses to PHA ConA and PPD before he entered therapy This agrees with the results of other investigators studying lymphocyte responses of patients with Wegeners granulomatosis (??) This condition shares many pathological features with polyarteritis nodosa (8) The frequency of lymphocytes binding SRBC was also subnormal in our patient However the peripheral blood picture and the serum Ig levels were essentially normal Thus the cellular immunity of the patient was suppressed before treatment It is also possible that the humoral immunity was suppressed since antibodies against a large number of viral antigens including herpes simplex and cytomegalovirus could not be demonstrated These negative results could possibly be due to the presence of RF (24) although we were unable to detect elevated levels of such factors in our patient s sera

Treatment resulted in a rapid increase in the reactivity of the lymphocytes to PHA and ConA and the frequency of E cells was normalized The latter effect may not have been due to an increased frequency of T cells but rather to a better expression of their SRBC receptors Some PPD reactivity was also noted after treatment The frequency of eosinophils increased after the start of treatment This finding however is probably not related to the patient s vasculitis since a similar effect is noted in patients with penile carcinoma receiving identical treatment (4) The serum levels of IgA and IgG increased at the end of the treatment The data above indicate that the bleomycin treatment largely corrected the immunological deficiency of the patient It is unlikely that the radiotherapy had contributed to any significant extent since it was directed against a very small part of the body Moreover radiotherapy is a well known immunosuppressor (5 13 16 20)

The overall impression is that bleomycin strongly activated the immune system of the patient One possibility is that this drug killed or inactivated inhibitor cells which were immunosuppressive The relatively benign course of the vasculitis before treatment may have been due to the immunosuppressed state of the patient At present we cannot exclude the possibility that the bleomycin treatment which evidently improved the general immunological competence of the patient enhanced the disease process which is probably of an immunological nature

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Cytomegalovirus Pneumonia after Treatment with Melphalan and Prednisone

Report of a Case

Finn von Eyben and Tom Skyhøj Olsen

From Department of Internal Medicine F and the Institute of Pathology Glostrup Hospital Glostrup Denmark

ABSTRACT A patient with multiple myeloma had pancytopenia after treatment with melphalan and prednisone and died of an interstitial pneumonia Post mortem examinations showed cytomegalic cells in the lungs Lung tissue showed a high titer of cytomegalovirus Only when other causes have been ruled out by microbiologic, serologic, and histologic examinations should melphalan be believed to cause respiratory illness

Two cases of fatal respiratory illness considered to be caused by melphalan have been reported (1 7) The following is a case of fatal cytomegalovirus pneumonia after treatment with melphalan and prednisone

CASE REPORT

A 51 year old man with multiple myeloma was given cyclophosphamide from Dec 1974 to July 1975 When a hemorrhagic cystitis appeared treatment with cyclophosphamide was discontinued From Aug 1975 the patient received melphalan 2 mg a day

Multiple lymphomas developed during Sept 1975 A lymph node biopsy showed dominance of plasma cells The patient was treated with prednisone 10 mg 2–4 times a day from the middle of Sept At the end of Sept the lymphomas were treated with low dose involved field X ray From the middle of Sept WBC and platelet counts became increasingly lower The patient went into hospital and treatment with melphalan was discontinued except on four days at the end of Nov From the beginning of Dec his temperature rose (from 37 8 to 39 8 C) and he was coughing and expectorating The sputum did not contain any blood Percussion and breath sounds were normal Treatment with nystatin penicillin ampicillin gentamicin cephalothin trimetoprim sulphamethoxazole carbenicillin blood transfusions gammaglobulins and nasal oxygen did not prove successful The patient became increasingly dyspneic and died

Investigations

Laboratory tests Shortly before the patient died his Hb was 87 g/l (normal range 140–175) WBC $0\ 2\times10^9$ cells/l (normal range 4×10^9–10×10^9) and platelet count 4×10^9 (normal range 200×10^9–500×10^9)

Chest X rays Before Aug 1975 the chest X rays were normal From Oct they showed a diffuse interstitial pneumonia Towards the end of Nov an abscess appeared in the left lung and the interstitial pneumonia progressed (Fig 1)

Microbiologic examinations During his final stay in hospital blood cultures and a urine culture did not show growth of bacteria Three tracheal aspirates showed usual



Fig 1 Diffuse bilateral interstitial pneumonia and abscess in the left lung during final hospitalization

ANNOUNCEMENTS

Eleventh Miles International Symposium The Mechanisms of Pain and Analgesic Compounds will take place in Turner Auditorium Johns Hopkins Medical Institutions Baltimore Maryland USA June 7–9 1978

Further information E G Bassett Ph D, Miles Laboratories Inc Elkhart Indiana 46514 USA

G v Hevesy Prize for Nuclear Medicine amounting to US$ 10000 will be awarded in memoriam of Georg von Hevesy who was honoured with the Nobel Prize in 1943 at the opening session of the *World Congress of Nuclear Medicine* in Washington D C on Sept 17 1978

Unpublished papers preferably in English but accepted also in German and French dealing with nuclear medicine may be submitted by July 1 1978 to Prof Dr W Horst University Clinic for Nuclear Medicine Raemistrasse 100 Ch 8091 Zurich Switzerland A committee will decide upon the award having the option of splitting the prize The authors should not have accomplished their 45th year of age by Sept 17 1978 and the manuscript should not exceed 9 typewritten pages

The Second World Congress of Pain will be held at the Queen Elizabeth Hotel in Montreal Canada Aug 27–Sept 1 1978

Enquiries Secretariat Second World Congress on Pain 3587 University Street Montreal Quebec H3A 2B1 Canada

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EDITORIAL

The Role of the Kidney in the Pathogenesis of Essential Hypertension

The development of hypertension in genetically hypertensive rats has been shown to be associated with sodium retention (2) The kidneys of hypertensive rats excreted less sodium than normotensive rats when perfused at the same pressure (12) The dominant renal hemodynamic feature of the spontaneously hypertensive rat has been found to be an increased preglomerular resistance caused by structural vascular changes in the preglomerular arterioli resulting in an increased glomerular filtration pressure (6) Thus there are animal data suggesting that the ability of the kidneys to excrete salt and water is hampered in at least some of the animal models of human essential hypertension

What is the evidence for a role of the kidney in *human* essential hypertension? The kidney could play a role either via abnormal production of hormonal substances such as renin angiotensin kinins and prostaglandins or via changes in the handling of salt and water Plasma levels of renin and angiotensin II are extremely high in malignant hypertension but this is not the case in uncomplicated benign hypertension The activity of the renin–angiotensin system even seems to be decreased in a substantial proportion of hypertensive patients Thus although several questions remain unsolved the burden of evidence does not point towards a primary role for the renin–angiotensin system in the development of essential hypertension

Abnormalities in sodium balance have been suspected on theoretical grounds and from animal data (7 9) but until recently differences in urinary sodium excretion have not been detected between normotensives and hypertensives This has probably been due to the fact that only mean values have been compared or that when regression analysis has been done between blood pressure and urinary sodium excretion the relationship has been hidden by problems of urine collection The noise caused by the large variance of a single casual BP is also important Recent Scandinavian studies performed on unselected middle aged normotensive and hypertensive men (1 13) have shed new light on the relationship The former study using a highly standardized method for recording resting BP and a detailed written and verbal information to avoid collection errors showed a non linear relationship between resting BP and sodium excretion Up to the level of 90 mmHg in resting diastolic pressure sodium excretion rose in agreement with the Guyton theory of pressure diuresis (7) Above 90 mmHg however sodium excretion fell as BP rose These observations have recently been verified by several investigators (8 10 13) though the latter two only deal with the normotensive part of the population

Assuming steady state conditions sodium excretion reflects salt intake If the Scandinavian data hold true this could mean that salt appetite increases with BP in the normotensive population and decreases with BP in the hypertensive population How does this fit with the widely held view that salt intake in the industrialized part of the world is mainly determined by habit or even addiction? Is there a plausible physiological explanation for the findings? With regard to the first question there is scarce evidence for the view that the large variation in salt intake within and between populations is the result of differences in the habitual use of salt The main argument *for* the view has been that salt intake in an industrialized country exceeds the bodily need to such an extent that some form of addiction to salt must have taken place presumably way back when salting was the main method of preservation Although intriguing this view is merely based on a series of assumptions and has not been substantiated with hard data

Is there a plausible physiological explanation for the non linear relationship between BP and sodium excretion=salt intake? In animal experiments angiotensin II and reduction of extracellular volume have been shown to be the strongest stimulators of salt appetite (11) As mentioned above in genetically hypertensive rats the development of hyper

tension has been shown to be associated with a mild sodium retention (2) This retention has also been found to occur in the early phase of hypertension in spontaneously hypertensive rats (4) Thus a possible explanation for the non linear relationship between BP and sodium excretion might be a gradually increasing difficulty for the kidney to excrete sodium as hypertension develops leading to mild salt retention and increase of the extracellular volume with secondary reduction of the appetite for salt As a matter of fact in the more severe stage of hypertension when sodium excretion (=salt intake) is low plasma renin activity (and hence angiotensin II) has been shown to be lower (3) than in earlier phases of hypertension when salt intake is high Taken together these data suggest that the *renin–angiotensin system might be the mediator of* salt appetite also in man However much work remains to be done before we know how salt appetite is mediated in man and how it is involved in the pathogenesis of essential hypertension One thing is *clear we cannot continue to assume that the* amount of salt we consume daily is merely a reflection of cultural and social patterns Salt intake is very c osely guided by physiological mechanisms in imals and it would be surprising if the human y with all its refined regulatory mechanisms s not use them to monitor the intake of salt one of the major determinants of milieu interieur

Can the two views on the determinants of salt intake be brought together? One explanation that takes most facts into account would be that the *level* of salt intake in the population is mainly determined by cultural habits while within the population the variation in salt intake is caused by differences in the mechanisms regulating sodium appetite discussed above Within a population the genetic susceptibility to develop hypertension due to difficulties in excreting a sufficient amount of sodium to retain normotension should be normally distributed if one buys the hypothesis of polygenic inheritance If populations with similar genetic susceptibility are fed with different loads of salt varying prevalences of hypertension increasing with increase in salt intake would be found if impaired renal ability to excrete sufficient sodium were the main mechanism by which hypertension develops An increase in the prevalence of hypertension with increasing intake of salt has been found in many studies (5) Those who develop hypertension due to this inherited difficulty to excrete sufficient sodium would retain some sodium which would increase extracellular volume and blood pressure decrease the activity of the renin–angiotensin system and secondarily decrease sodium appetite

Goran Berglund Gothenburg Sweden

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Cytostatic Treatment of Glomerular Diseases

IV *The Effect of Combined Immunosuppressive Treatment on Serum Creatinine and Proteinuria Evaluated by Sequential Statistical Analysis*

Report from a Copenhagen Study Group of Renal Diseases

J T Balsløv C Brun F Jørgensen H E Jørgensen
M Larsen S Larsen I Lorenzen and Å C Thomsen

From the Department of Medicine Blegdamshospitalet Departments of Clinical Chemistry and Medicine III Kommunehospitalet Medical Department B Bispebjerg Hospital Medical Department B Glostrup Hospital and the Department for Data Processing in Medicine and Medical Department C Gentofte Hospital Copenhagen Denmark

ABSTRACT Sequential statistical testing of the development of the disease in the single patient was used in the assessment of immunosuppressive treatment of renal glomerular diseases After an initial period of prednisone (P) treatment, this was supplemented first by azathioprine (A) and later in addition by cyclophosphamide (C) The time of transition from one treatment to another was determined by the result of the current statistical testing of the correlation between serum creatinine concentration and proteinuria on the one hand and the time and the varying doses of the drugs on the other Twenty nine patients entered the study Thirteen were withdrawn, eight for technical reasons, three due to side effects and two on account of renal deterioration and transfer to dialysis treatment Eight patients were cured, one during P treatment, five during P + A, and two during the combination of P, A and C Eight patients completed treatment without being cured In the overall material no statistically significant change in serum creatinine was noted whereas the proteinuria decreased during P + A and P + A + C No dose dependent therapeutic effect of the drugs was demonstrated In conclusion, this combined treatment with P, A and C did not seem to yield any major therapeutic progress The technic of sequential statistical testing may be a useful tool in clinical research

The proper role of cytostatics in the treatment of renal glomerular diseases is still unsettled despite several controlled clinical trials

Azathioprine seems to have a favourable influence on proliferative glomerulopathy in lupus nephritis (6) but the effect is only marginally better than with prednisone alone (8 10) On the whole the evidence for any effect of azathioprine on glomerular diseases is scanty (1 14 16 19) Cyclophosphamide on the other hand has proved its effect in controlled trials in a number of conditions including frequently relapsing nephrotic syndrome in children with minimal changes or proliferative glomerulopathy (4 7 12) and other types of glomerulopathy in adults (2 18) In general however the results from cytostatic treatment of glomerular diseases have been unsatisfactory The effect of cyclophosphamide compared to that of prednisone alone in lupus nephritis is doubtful (8 9)

In 1971 Mukherjee (15) reported that a combined immunosuppressive treatment with prednisone azathioprine and cyclophosphamide was able to bring about a complete remission in 10 out of 12 patients with proliferative glomerulopathy resistant to treatment with prednisone and azathioprine Brown et al (5) also reported a favourable effect of a combined drug treatment of extracapillary glomerulonephritis

The purpose of the present investigation was primarily to analyse the variations in serum creatinine and proteinuria during a stepwise established combined treatment with prednisone, azathioprine and cyclophosphamide in patients with glomerular diseases and to describe a possible relationship between the doses drugs

Table I *Initial values of serum creatinine concentrations and proteinuria in relation to clinical diagnosis and to type of glomerular lesions*

	No of pats	Serum creatinine (mg/100 ml)			Proteinuria (g/24 h)			Age range (y)
		Mean	S D	Range	Mean	S D	Range	
Clinical diagnosis								
Connective tissue diseases	16	1 56	0 85	0 5–3 7	3 49	2 87	0 1–10 9	8–69
Glomerulonephritis	12	1 74	1 19	0 6–4 6	3 03	2 09	0 2–8 7	11–72
Idiopathic nephrotic syndrome	1	1 0			12 3			61
Type of glomerular lesion								
Minimal changes	2	1 10	0 14	1 0–1 2	7 47	6 85	2 6–12 3	
Proliferative	13	1 28	0 66	0 6–3 0	1 70	1 38	0 1–5 5	
Lobular	4	1 96	1 02	0 5–2 9	3 79	0 35	3 4–4 2	
Extracapillary	1	2 4			4 1			
Epimembranous	1	0 5			2 7			
Membranoproliferative	3	1 25	0 25	1 0–1 4	6 11	2 80	3 1–8 7	
Segmental focal	1	1 2			2 8			
Unclassifiable	4	2 79	1 64	1 3–4 6	6 10	3 78	3 0–10 9	

effects on serum creatinine and proteinuria Secondarily the purpose was to examine the applicability of a clinico statistical design developed to measure sequentially the short term response to treatment in the individual patient

PATIENTS AND METHODS

Definition of the patients

Admission of patients to the trial was based on the following criteria 1) Histological glomerular changes 2) At least one of the following signs (*a*) decreased glomerular filtration rate as estimated by creatinine clearance (*b*) proteinuria (>200 mg/24 h) (*c*) pathological urinary sediment on microscopy Excluded from treatment were patients with glomerular diseases caused by diabetes mellitus amyloidosis arterial hypertension or pyelonephritis as well as children with idiopathic nephrotic syndrome and non cooperative patients

This histological and clinical classification was as presented previously (3)

Laboratory investigation

The glomerular filtration rate was estimated by serum creatinine and 24-hour endogenous creatinine clearance Quantitative determination of urinary protein excretion was carried out as mentioned elsewhere (3) The investigations were performed before and at 2 week intervals during treatment The haematological state (Hb thrombocytes leucocytes and differential count) was controlled at 1 and 2 week intervals

Statistical design

Values of serum creatinine and proteinuria and doses of the drugs for the individual patient were currently registered Serum and urine specimens were examined and dose levels of drugs were changed at random every second week At the same intervals sequential statistical analyses were made by means of a computer program system in order to reveal dependency between dose and effect and between time (duration of treatment) and effect At least four and at most 11 analyses were used in a sequential rank correlation test and with error probabilities of 15% (see Appendix)

The duration of the individual treatment regimes depended upon the result of the statistical analyses At the time when the sequential analyses allowed a decision (cf the section on treatment below) a new treatment schedule was started In this way patients acted as their own controls

The statistical results for the individual patients were collected in a material from the whole patient population as in common clinical trials

Treatment

The treatment was given in the following sequence and doses 1) Prednisone 0 3 mg/kg b wt /24 h for four weeks 2) Prednisone plus azathioprine The dose of each drug was changed at 2 week intervals in a random way The dose of prednisone varied from 0 1 to 0 6 mg/kg b wt /24 h and of azathioprine from 1 0 to 3 5 mg/kg b wt /24 h This combination was continued until two of the combinations of variables (time and/or dose of drug versus proteinuria and/or serum creatinine) presented a statistically significant result in the sequential analysis If these two combinations showed a lack of correlation the result from a third pair of variables was awaited The second treatment period lasted for 4–11 two-week periods 3) Prednisone plus azathioprine plus cyclophosphamide Azathioprine was given in a fixed (low) dose (1 0 mg/kg) to avoid the risk of giving azathioprine and cyclophosphamide in high doses at the same time The dose of cyclophosphamide varied between 0 30 and 2 0 mg/kg b wt /24 h The dose of prednisone varied as in period 2

Table II *Course of 29 patients in relation to the periods of treatment*

P=prednisone PA=prednisone+azathioprine PAC=prednisone+azathioprine+cyclophosphamide

Cause of exclusion	No of pats excluded during period of treatment P	PA	PAC	Total
Cured during treatment	1	5	2	8
Deterioration of renal disease		2		2
Side-effects		3		3
Technical reasons	1	6	1	8
None completed treatment			8	8
Total	2	16	11	29

The duration of this period varied in the same way as in period 2 The treatment was definitely terminated when the patient had been treated with all three drugs and the analysis had given a statistically significant result as mentioned above The treatment was likewise discontinued if the patient was cured i e no proteinuria, normal urinary sediment, and normal creatinine clearance for at least four weeks For all three drugs six different dose levels were used (except for azathioprine in period 3) To avoid accidental correlations between the doses of two drugs used at the same time or between time and the dose of an individual drug, dose regimens based on computer generated random numbers were applied

The sequence of drugs put into play in the present study was motivated by the greater gonadal toxicity of cyclophosphamide

RESULTS

Description of the patients

A total of 29 patients entered the study 17 males aged 11–72 years and 12 females aged 8–72 years

The clinical and histological diagnoses and initial values of serum creatinine and proteinuria are given in Table I

During the treatment period 18 patients were excluded for the reasons given in Table II and only 11 patients reached the cyclophosphamide period

Eight patients were cured as defined by the criteria given above The initial values of serum creatinine and proteinuria were statistically significantly lower in the group of cured patients than in the remainder (fixed sample size test $p<0.05$) The differences in clinical and histological diagnoses were not statistically significant The treatment was discontinued in two patients during the azathioprine period due to rapid deterioration of kidney function and both were transferred to dialysis treatment Three patients were withdrawn on account of side-effects all of them in the azathioprine period The causes were leucopenia and severe exacerbation of a chronic bronchitis thrombocytopenia and leucopenia and leucopenia complicated by an upper respiratory tract infection caused by staphylococci All three patients survived No other severe haematological disturbances were seen

Eight patients were excluded from the study for technical reasons most often lack of cooperation

Eight patients went through the full treatment schedule and a further three reached the final period of three-drug treatment These 11 patients did not differ from the remaining 18 in respect of initial values of serum creatinine and proteinuria.

The sequential analysis based on individual patients

Table III shows the distribution of the results of the individual sequential statistical tests in relation to the possible influence of time (duration of the treatment) and doses of the drugs given with respect to changes in serum creatinine and proteinuria As all the statistical tests are based on the same hypotheses and have the same probabilities of error a comparison of the sums is permissible

Three tendencies emerge from Table III First serum creatinine did not decrease during the azathioprine (plus prednisone) period Second proteinuria did not increase during the same period Third during the cyclophosphamide treatment the dose of prednisone did not correlate negatively with proteinuria These results might be explained by chance However a supplementary non-sequential quantitative analysis confirmed the statistical significance of the last two statements while the first statement concerning serum creatinine seemed purely incidental Thus only one short term dose dependent effect of the drugs given could be demonstrated namely an unfavourable effect of larger doses of prednisone during cyclophosphamide treatment

Analysis based on the material as a whole

The variations in average values of serum creatinine and proteinuria from period to period are shown in Figs 1 and 2

Table III *Number and distribution of results from sequential statistical tests performed on individual patients*

PA=prednisone + azathioprine PAC=prednisone + azathioprine + cyclophosphamide

Period of treatment	Variables with possible influence	Correlation[a]	Dependent variables: S creatinine	Proteinuria	Relative increase in: S creatinine	Proteinuria
PA	*Duration of treatment (time)*	+	8	4		
		0	7	3		
		−	1	9		
	Prednisone (dose)	+			0	3
		0			12	9
		−			1	1
	Azathioprine (dose)	+			1	3
		0			12	9
		−			1	2
PAC	Duration of treatment (time)	+	1	2		
		0	8	5		
		−	2	4		
	Prednisone (dose)	+			0	5
		0			7	4
		−			2	0
	Cyclophosphamide (dose)	+			0	1
		0			8	8
		−			2	1

stically significant rank correlation tests
itive −=negative correlation 0=no significant correlation

The changes found in average levels of serum creatinine were not statistically significant On the other hand the level of proteinuria was lower in the azathioprine (+ prednisone) period than during prednisone treatment alone and it was still lower in the cyclophosphamide period This last decrease could not be explained by the slight rise in serum creatinine in the same period

The courses of serum creatinine and proteinuria within the individual treatment periods were as follows serum creatinine did not change statistically significantly whereas proteinuria decreased during azathioprine treatment The same holds true when all three treatment periods are considered as a whole, and also if the analysis comprised only the 11 patients who entered the cyclophosphamide period

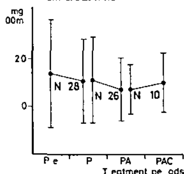
Side effects

Three patients developed leucopenia during the azathioprine period that necessitated discontinuation of the treatment One of these patients also showed severe thrombocytopenia Transient slight alopecia was observed in most of the patient receiving cyclophosphamide No deaths were ascribed to the cytostatic treatment

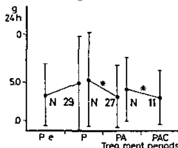
DISCUSSION

In the present study of combined immunosuppressive treatment with prednisone azathioprine and cyclophosphamide we were unable to confirm the favourable effects reported by Mukherjee (15) in glomerulonephritis As appears from Table II only two of the 11 patients reaching the stage of combined treatment with prednisone azathioprine and cyclophosphamide were cured during this regimen Our observations suggest that treatment with prednisone plus either azathioprine or cyclophosphamide might have led to same result as the treatment with all three drugs in the present study

The treatment regimen probably had some therapeutic effect Disregarding the eight patients who were excluded from the trial for technical reasons 38% (8/21) of the patients were cured This is close to the 42% (13/31) of patients who



F g 1 Mean+1 S D of serum creatn ne concentration n the nd dual treatment penods *N* no of pat ents part c pat ng n both penods Abbrev at ons penods as n Table II



F g 2 Mean+1 S D of prote nuria n the nd v dual treat ment penods * Presence of a stat st cally s gn f cant change from one penod to another ($p<0.05$) Abb evia t ons as n F g 1

normalized or mproved in our previous controlled study of four months cyclophosphamide treatment of glomerular d seases (2)

Analys s of the total number of pat ents—as well as analysis of only the 11 pat ents rece v ng all three penods of *treatment—revealed a decrease in pro*te nuria during the azath oprine period wh ch con t nued in the cyclophospham de period Th s de crease was not caused by a fall ng glomerular filtra t on rate

The lack of a demonstrable dose dependent therapeut c effect could be explained n several ways The drugs might have an equal therapeut c effect at all the dose levels selected the effect of one dose level might extend beyond the period in wh ch the dose was given and two weeks m ght be too short a period to reveal s gnif cant changes n prote nuria and serum creat n ne Steinberg et al (18) found a correlation between the dose of cyclophospham de and the degree of mprovement n a s udy of ten weeks treatment of lupus glomerulonephritis McCrory et al (13) reported the same effect of 5 mg cyclophospham de/kg g ven n 45 days as of 2 5 mg/kg in 90 days in treatment of children with frequently relapsing nephrot c syndrome with min mal les ons

Our cl n co stat st cal procedure of analys s was developed for the present study and seems to offer certa n advantages The techn c allows sequential analys s of an extensive range of data n the course of the d sease n the ind vidual pat ents It is possi ble to analyse the development of variables in rela tion to one another and to time and therapy Fur ther it is poss ble to compare the results of the se*quent al analysis from several patients* n a sec ond step (sequent al or non sequential analysis) Analysis of results from only a few patients fol lowed continuously will allow stat stical conclu s ons with very small error probab l ties The method permits a l m ted duration of the period of *invest gat on as well as an early recognition of er* rors in the collection of data n cl nical trials

APPENDIX

Sequent al rank co relat on est

Ghosh (11) has given a sequent al probab lity rat o test for the correlat on coeff c ent of b variate normal distribu t ons The stat st c s $Z\ f(r\ \varrho\ n)/f(r\ \varrho_0\ n)$ where r is the sample correlat on coeff c ent ϱ_0 and ϱ are the theoretical correlat on coeff c ents accord ng to the hypotheses H_0 and H respec vely and n s the number of bivariate observations $f(r|\varrho\ n)$ s the frequency function of r which can be calculated rather unproblemat cally on a computer

The Wald approx mate dec s on lim ts can be used H_0 is accepted when $Z\leq\beta/(1\ \alpha)$ H s accepted when $Z\geq(1\ \beta)\ \alpha$ and sampl ng s continued if $\beta/(1\ \alpha)< Z<(1\ \beta)/\alpha$ whe e α s the le el of the test and $1\ \beta$ is the power

S mulat ons have shown that the statistic Z can also be used when the Spearman rank correlation s subst tuted for r However we are then no longer deal ng w th normal d stribut on correlations ϱ_0 and ϱ

The result of a test s a b nomial event wh ch can n turn be ut lized n a second step sequential analys s

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The Epidemiology of Febrile Reactions in Haemodialysis

Hans Jørn Kolmos and Susanne Møller

From the Department of Nephrology and Statens Seruminstitut Regional Laboratory Odense University Hospital Odense and the Biostatistical Department Statens Seruminstitut Copenhagen Denmark

ABSTRACT Febrile reactions were studied in 2000 consecutive haemodialyses performed in 85 patients A number of 219 febrile reactions were registered in 49 patients (11%) The overall month to-month incidence showed little variation Febrile reactions were not distributed randomly among the patients those with respiratory tract infection experienced more febrile reactions during periods with infection than during periods without Similarly, the incidence was higher in patients with than without chronic urinary tract infection A low incidence was registered both in patients under 40 years of age and in patients having had more than 100 dialyses at the beginning of the observation period The frequency was the same whether single pass or recirculating single-pass monitors had been used, and it was not influenced by blood transfusions during dialysis Thus our analysis leads to the conclusion that the majority of the febrile reactions registered among the present patients were determined by endogenous factors such as infection, while exogenous factors such as dialysate bacteria and pyrogens seem to have played only a minor role

Febrile reactions are one of the major complications in patients undergoing maintenance haemodialysis (8) According to the prevailing opinion they are caused by the passage of dialysate bacteria and pyrogens through the dialysis membrane into the blood stream (1 3 4 5 7 8 10) On the other hand it has been found that patients with infections develop fever during dialysis (9) thus suggesting an endogenous rather than an exogenous origin

This study was originally designed to evaluate the influence of exogenous factors on the occurrence of febrile reactions during haemodialysis using the single dialysis as the unit of statistical calculation However it soon became evident that the incidence was not the same for all patients All analyses had therefore to be performed with *the patient* as the statistical unit and a method applicable for this purpose had to be developed

The study describes the epidemiology of febrile reactions including the following parameters time distribution type of dialysis monitor blood transfusion during dialysis sex and age previous dialytic treatment and presence or absence of respiratory and urinary tract infections An account is given of the statistical method worked out

Requests for reprints to H J Kolmos M D Department of Nephrology Odense University Hospital DK 5000 Odense Denmark

PATIENTS AND METHODS

The study includes 2000 consecutive haemodialyses performed in the Haemodialysis Centre at Odense University Hospital from Feb 23 to Sept 15 1973 Eighty five patients (49 females 36 males) 15–71 years of age received treatment The number of dialyses performed in each individual ranged from one to 65 Seventy-eight patients representing 1955 dialyses suffered from chronic renal failure and six patients representing 44 dialyses from acute renal failure One patient was dialysed once because of drug poisoning

The dialysis monitors were either the single pass type (DDS De Danske Sukkerfabrikker) or the recirculating single pass type (Travenol RSP Dasco and a Danish test model) All dialysers were disposable units (Gambro-Lundia Ultra Flo and Dasco) The dialysis fluid was delivered in sterile and ready mixed form However later investigations have demonstrated a significant proliferation of microorganisms in the monitors during dialysis (6) The temperature of the dialysate in the monitors was 38 0°C Heparinization took place with an initial dose of 5000 U administered directly into the tubing followed by doses of 2000 U depending on the coagulation time tested at intervals of 30–45 min The dialyses lasted for 1–10 hours

Body temperature was registered before and after

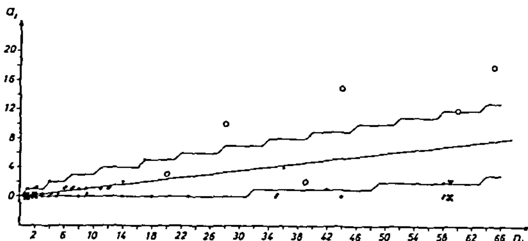


Fig 1 Number of febrile reactions and total number of dialyses in each of the 85 patients n_i=No of dialyses *received by the ith patient a_i=no of febrile reactions* among these n_i dialyses The lines illustrate the relations $a_i=p\times n_i$ (p=0 11 the total frequency of febrile reactions) and the 95% confidence limits for p=0 11 Points on the two lines and those lying in the area between *belong to the confidence interval O=Six patients with* accumulations of febrile reactions ●=the remaining 79 patients

dialysis *A febrile reaction* was defined arbitrarily as a rise in temperature during dialysis of at least 0 5°C and a rectal or axillary temperature after dialysis of at least 38 0 or 37 5°C respectively

Bacteriological examination of the urine was carried out least once a month in all cases The criterion for urinary infection was the isolation of the same bacterial ies with the same sensitivity pattern from at least two consecutive cultures in concentrations of at least 10^5/ml urine The criterion for respiratory tract infection was the simultaneous occurrence of fever characteristic physical signs and roentgenographical pulmonary consolidations

STATISTICAL METHODS

Febrile reactions were not distributed randomly among the patients (Fig 1) Therefore evaluation of the importance of factors concerning the *individual dialysis* (type of monitor blood transfusion and acute disease present only during some dialyses e g respiratory tract infection) had to be made *within* the patient i e each patient acting as his own control For this purpose only the febrile reactions of each patient that occurred independently could be used for the analysis The independence of the febrile reactions of each patient was *tested by a run test in which the changes (+fever/−fever/* +fever etc) were counted The test could be carried out only for patients with at least two febrile reactions i e 31 patients in six of whom the number of runs fell below the 5% significance level As their febrile reactions thus appeared in clumps these patients were excluded from the subsequent tests

The importance of the dialysis factors was evaluated by means of the χ^2 test or Fisher s exact test on the 2×2 table {±fever×±dialysis factor} within each patient observed both with and without the dialysis factor These tests were supplemented by a sign test based on a record for each patient of whether the frequency of febrile reactions was highest with or without the dialysis factor Respiratory tract infection proved to be the only factor which significantly affected this frequency

The importance of factors concerning the *individual patient* (sex age and chronic disease such as urinary tract infection) could be evaluated *between* patients only after each patient had been allotted a characteristic for the frequency of febrile reactions in which allowance had been made for the effect of respiratory tract infection Therefore the dialyses were divided into two groups according to the presence or absence of respiratory tract infection (+RTI −RTI) Within each of these groups an

Table I *Grouping of patients according to fever characteristics during periods with (+RTI) and without respiratory tract infection (−RTI)*

x=Patients in the +RTI group only y=patients in the −RTI group only

Fever characteristics during periods in +RTI	Fever characteristics during periods in −RTI						Total
	1	2	3	4	5	x	
1							0
2		1	2			1	4
3		2	4	1	3	1	11
4			2	2	1		5
5				1			1
y	8	14	22	11	3		58
Total	8	17	30	15	7	2	79

Table II *Distribution of time of dialysis among patients with and without febrile reactions*

Week no	+fever	−fever	% fever
1–4	31	244	11.2
5–8	43	232	15.6
9–12	32	246	11.5
13–16	28	239	10.5
17–20	27	239	10.2
21–24	19	239	7.4
25–29	39	342	10.2
Total	219	1781	11.0

Table III *Distribution of dialyses with and without febrile reactions among the monitors used*

Monitor		No of units	Dialyses		
Mark	Type		+fever	−fever	% fever
Travenol	RSP	5	107	856	11.1
DDS	SP	5	90	664	11.9
Dasco	RSP	2	8	112	6.7
Test model	RSP	1	14	149	8.6
Total			219	1781	11.0

RSP=recirculating single pass SP=single pass

approximative χ^2 test was performed in order to see whether the patients had a common frequency of fever. In the −RTI group with the low total frequency, 25 patients with less than five dialyses were excluded from this test in order not to overestimate the significance. There were 19 patients with dialyses in both the +RTI and the −RTI group, two in the +RTI group only and 58 in the −RTI group only.

In order to select those with many and those with few febrile reactions the following probabilities were calculated for all patients in both groups based on the total frequencies (39.0% and 7.6%, Table IV). $p1$=the probability of observing r or fewer febrile reactions among n dialyses, $p2$=the probability of observing r or more febrile reactions among n dialyses, where r is the observed number of dialyses with febrile reactions and n the total number of dialyses.

The patients were classified into five groups according to the frequency of fever (fever characteristics): 1. $p1<0.025$ (significantly lower than expected), 2. $p1<0.500$ (lower than expected, not significant), 3. $p1>0.500$, $p2>0.500$ (as expected), 4. $p2<0.500$ (higher than expected, not significant), 5. $p2<0.025$ (significantly higher than expected). The classification is shown in Table I. Within the −RTI group a χ^2 test was used to see whether there was any difference in the distribution of fever characteristics between the 19 patients who also belonged to the +RTI group and the remaining 58 patients. As regards the 19 patients, there was in no case a conflict between the fever characteristics in the +RTI and −RTI groups, and the patients were allotted the most extreme characteristic (Table V). Using the patient as unit, the relationship between the fever characteristic and the other factors concerning the patient was evaluated by χ^2 tests.

RESULTS

Altogether 219 febrile reactions were registered among 49 patients, the total incidence being 11%. Fifty one febrile episodes were associated with an initial rectal temperature of 37.5 C or more, and in 171 cases the rise in temperature during dialysis was at least 1.0°C.

Table II shows the relatively even distribution of febrile reactions during the course of the study. Febrile reactions were not distributed randomly among the patients, since 22 had either more or fewer febrile episodes than expected at the 5% significance level (Fig. 1, $p<0.0005$). Six patients showed periodic accumulations (Fig. 1).

Table III shows the distribution of febrile reactions among the dialysis monitors used. No major variations were found. The fever risk of the two most frequently used dialysis monitors (Travenol and DDS) was studied by analysing the fever incidence of all patients without accumulation of febrile reactions who had had at least ten dialyses and at least three febrile reactions while using these monitor types. Fifteen patients who had been dialysed with both monitor types fulfilled these criteria. In six of these the incidence of febrile reactions was highest when Travenol was used, while in eight patients the incidence was highest when using DDS. A similar comparison was not possible for the other two monitor types because of too few observations (78 dialyses with Dasco and 98 with the test model were performed on patients who either experienced no febrile reaction or were dialysed with one monitor type only).

Blood transfusions coincided with febrile reactions in 14 out of 82 dialyses among patients observed during dialysis, both with and without administration of blood; this had no significant influence on the fever incidence.

Table IV shows the incidence of febrile reactions among the patients without accumulations during periods with and without respiratory tract infection. Sixteen of the 19 patients observed both with and without infection had the highest incidence when infected ($p=0.0044$). When not infected these 19

Table IV *Incidence of febrile reactions among the 79 patients without accumulations during periods with (+RTI) and without respiratory tract infection (−RTI)*

Incidence of febrile reactions	No of pats		No of dialyses +RTI	No of dialyses −RTI	Incidence of fever (%) +RTI	Incidence of fever (%) −RTI
Highest when infected	16	19	67	292	44.8	18.8
Highest when not infected	2					
Same whether infected or not	1					
Observed only when infected	2		15		13.3	
Observed only when not infected	58			1370		5.3
Total	79		82	1662	39.0	7.6

patients had the same distribution of fever characteristic as the 58 patients without respiratory tract infections (the distribution into characteristics 1–5 was 0 3 8 4 4 (sum 19) versus 8 14 22 11 3 (sum 58) Table I 0 10<p<0 20) but the incidence of fever was not the same for all patients in the −*RTI* group (approximative χ^2-test p<0 0001)

The consistency of the material made it possible by means of the statistical method described to allot each patient a fever characteristic which was essentially the same whether calculated from dialyses with or without respiratory tract infection (Table I)

Table V shows the incidence of febrile reactions among patients with and without chronic urinary tract infection in relation to the fever characteristics allotted in which allowance has been made for the influence of possibly co-existing respiratory tract infection Most patients with fever characteristics 1 and 5 had received a large number of dialyses while most patients with characteristic 3 had received only a few (19 out of 26 patients less than 6 dialyses) The table shows that there was a preponderance of patients with chronic urinary tract infection among those showing a high incidence of febrile reactions (χ^2 test on three groups viz. fever characteristics 1+2 3 and 4+5 p=0 03) although this does not explain the whole difference Even in the non-infected group febrile reactions were not distributed randomly since 8 (fever characteristics 1 and 5) out of 56 fell outside the 5% level (p<0 005) Furthermore 16 patients (fever characteristics 1 and 5) out of all the 79 patients had an incidence of febrile reactions that was either significantly lower or significantly higher than expected (p<0 005) thus demonstrating a non random distribution

A low incidence (fever characteristics 1 and 2) was more common among patients who had had more than 100 dialyses at the beginning of the observation period than among the remaining patients (15 out of 24 against 14 out of 55 p 0 0021)

Patients under 40 years of age had a low incidence more often than older patients (14 out of 24 against 15 out of 55 p=0 0089) There were no differences between males and females

Table V *Patients without accumulations grouped according to incidence of febrile reactions and presence (+UTI) or absence of chronic urinary tract infection (−UTI)*

Fever characteristics*	No of pats +UTI		No of pats −UTI		Total
1	3	6	5	23	8
2	3		18		21
3		5		21	26
4	7	12	9	12	16
5	5		3		8
Total		23		56	79

* Incidence of febrile reactions 1=significantly lower than expected, 2 lower not significant 3=as expected 4 higher not significant, 5=significantly higher

DISCUSSION

The non random distribution of febrile reactions observed among the patients in the present study corresponds with the results in previous reports (4 8) Our results indicate that acute respiratory tract infections and chronic urinary tract infections are predisposing factors The former was demonstrated by using each patient as his own control i e by comparing his different dialyses the latter by comparing the patients after each had been allotted a fever characteristic taking into account

the already established effect of respiratory tract infections

At the same time however infections are not the only endogenous factor of importance as is evident from the non random distribution of fever characteristics among patients without urinary tract infections

The pathogenesis of febrile reactions associated with infection may be as described by Schreiner and Maher (9) They found a decreased febrile response to infections in patients with severe azotaemia and suggested that febrile reactions during dialysis indicated the clinical manifestation of pre existing infections which were demasked as the azotaemia subsided This seems to be improbable as regards our patients since most of them were on maintenance haemodialysis and were thus well dialysed Alternatively we suggest that heparinization during dialysis is a factor of importance leading to a decapsulation of infectious foci with the liberation of pyrogens or microorganisms into the blood stream

The relatively constant month to-month frequency contrasts with the results of Curtis et al (1) and Jones et al (5) They registered epidemic accumulations of febrile reactions which were due to bacteraemia with the same dialysate bacteria and associated with inadequate disinfecting procedures during the preparation of Kiil dialysers Febrile reactions of this kind have not been observed with disposable dialysers

The same frequency of febrile reactions registered for the single pass and recirculating single pass monitors used in this study is remarkable since the latter type is known to be more heavily contaminated than the former (2 6) This indicates that the concentration of dialysate microorganisms is of little importance for the development of febrile reactions and contradicts the commonly held conception of a purely exogenous origin (3 7 10)

Our analyses lead to the conclusion that the majority of febrile reactions registered in this study are determined by endogenous factors such as infection while exogenous factors such as dialysate microorganisms and pyrogens seem to have played only a minor role

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Table I *Pertinent data on the patients*

ND=not done β=group A β hemolytic streptococci gln=glomerulonephritis

			At onset						At follow up		
Pat no	Age (y)	Sex	Throat culture	ASO	C3 in plasma (%)[a]	C4 in plasma (%)[b]	Maximal serum creatinine (μmol/l)	Hypertension	Serum creatinine (μmol/l)	Cr^{51} EDTA clearance (ml/min/1 73 m²)	C3 in plasma (%)
Exposed to organic solvents											
1	45	♂	ND	880	35	145	200	Yes	110	76	110
2	41	♂	ND	495	20	110	175	Yes	80	98	114
3	15	♂	β	1 380	15	144	300	Yes	70	82	84
4	12	♂	ND	2 400	25	120	470	Yes	65	ND	Normal[f]
5	10	♀	β	720	24	140	170	No	40	ND	Normal[f]
6	10	♂	β	800	17	80	60	Yes	50	ND	ND
7	51	♂	ND	2 500	27	119	170	Yes	80	93	120
8	55	♂	β	500	19	92	200	Yes	102	70	ND
9	14	♂	β	2 240	62	140	600	Yes	140	49	120
10[d]	36	♂							250	27	82
Not exposed to solvents											
11	61	♀	ND	750	36	56	1 250	No	120	ND	120
12	6	♂	ND	560	2 5	59	360	Yes	50	ND	Normal[f]
13	4	♂	ND	720	35	105	95	No	20	ND	131
14	4	♂	β	300	6	152	50	No	40	ND	135
15	5	♂	β	570	27	178	60	No	45	ND	124

[a] Normal range 70–140 [b] Normal range 53–207 [c] He=hematuria Hy=hypertension Pr=proteinuria [d] Not studied at onset [e] Skin culture [f] Judged by agarose gel electrophoresis of plasma [g] Biopsy done 3 years after onset 1 year after cessation of exposure

other 5 patients had apparently only insignificant or no earlier contact with solvents Table I gives the pertinent data on the patients

Four patients (nos 7 8 9 10) were again exposed to solvents after having been recovered from their acute symptoms At the last follow up 18 months to 3 years after onset 3 of these 4 patients had proteinuria and a low GFR (49 27 and 70 ml/min) and 2 (nos 9 and 10) were hypertensive In patient 7 the exposure after the acute nephritis was only mild

In 11 patients the exposure after recovery was at most insignificant None of them had hypertension or proteinuria at the after examination One patient (no 11) had an elevated serum creatinine level (120 μmol/l) and one (no 1) a slight decrease in the GFR (76 ml/min) 1 and 7 years respectively after the acute onset The other 9 patients had a normal GFR or a normal serum creatinine level when last seen 3 months to 2 years after the onset

None of the 15 controls reported exposure to solvents immediately before the infection with nephritogenic streptococci Three had had short episodes of mild to moderate exposure but these episodes were either several months after or several months or years before the infection Twelve controls had no or insignificant contact with organic solvents

ol ow p at	Symp- toms	Expo- sure after acute onset	Renal biopsy light and immunofluorescence microscopic findings
7 y	He	No	Lobular exudative gln
0 mo		No	ND
5 mo	He	No	Lobular exudative gln Parietal C3 properdin
5 mo	He	No	Lobular exudative gln
3 mo	He	No	ND
6 mo		No	ND
2 y	He	Yes	Lobular exudative gln Parietal IgG IgA IgM C3 Mesangial IgG IgA IgM C3 C4
8 mo	He Pr	Yes	Exudative gln
3 y	He Hy Pr	Yes	Focal glomerulosclerosis*
0 mo	He Hy Pr	Yes	Lobular proliferative gln
1 y	He	No	Extracapillary exudative gln Parietal IgG IgA Mesangial IgG IgA C1q C3 C4
2 y		No	ND
1 y		No	ND
2 mo	He	No	ND
3 mo		No	ND

CASE REPORTS

(Supplementary data are given in Table I)

Histories of 6 patients who reported brief exposure shortly before the onset of glomerulonephritis

Case 1

This 45-year-old customs officer had a sore throat and fever in Dec 1969 and stayed at home where the apartment including the room in which he was lying was being painted and the air was constantly heavy with vapours of the paint solvents After about 14 days his arms legs and face swelled and his urine output decreased He was admitted to hospital in late Jan 1970 presenting a nephrotic syndrome

Case 2

This 41 year-old musician had been exposed to solvents twice in his life The first time was at 20 years of age when he painted scenes for 3 or 4 days On the last day he had gross hematuria. In Feb 1974 he had an acute myocardial infarction In early Nov he started painting his kitchen This work took about two weeks During this time he lost his sense of taste and smell and was nauseated because of the smell of the paint On Nov 19 he had fever and a sore throat Fifteen days later he was admitted to hospital becuase of symptoms and signs of acute glomerulonephritis

Case 3 (Fig 1)

This 15-year-old boy had fever and a sore throat on Aug 23 1976 He recovered after 2–3 days On Sept 5 he visited a friend who was spray painting a car in a small unventilated room This work took about 1 hour but the boys stayed in the garage for 3 hours The following morning he had severe headache vomited and observed that his urine was dark brown On admission to hospital the same day he presented a full blown acute glomerulonephritis

Case 9

This 14-year-old boy began his hobby of constructing plastic models in Aug 1973 His first symptoms appeared one month later He spent at least one hour a day and entire week-ends working on the models He used a glue solved in trichloroethylene His maternal grandfather had died of uremia and his grandmother suffered from proteinuria and anemia. On Sept 22 he developed pain in his right foot A few days later he had lumbar pains as well and on Sept 29 he had gross hematuria which lasted for a few days He afterwards felt tired and suffered from a sore throat About 10 days later the hematuria recurred and he was admitted to hospital with symptoms and signs of an acute glomerulonephritis In March 1974 his serum creatinine was 80 μmol/l He discontinued his hobby in the late spring of 1975 In May 1976 the serum creatinine was 140 μmol/l and the GFR 49 ml/min his urine contained moderate amounts of protein and occasional erythrocytes and his BP was 145/100 mmHg On that occasion renal biopsy was performed

Case 5

This 10-year-old girl had a sore throat and fever on Jan 13 1975 She was treated with penicillin for 10 days On Jan 30 she became ill again with fever cough and hoarseness During most of Jan her father was painting the basement which she occasionally visited but never stayed there very long At the same time the girl had started woodwork at school and on several occasions she painted in a small room She never felt discomfort and the total duration of exposure at home and at school was short probably not more than a few hours On Feb 8 she had gross hematuria and was admitted to hospital with symptoms and signs of acute glomerulonephritis

Case 6

This 10-year-old boy had frequent attacks of headache On May 15 1975 a neighbour started to paint a wooden

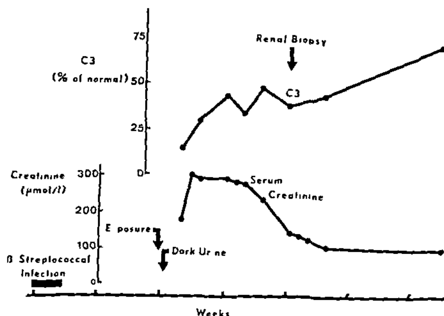


Fig 1 Course in patient 3

terrace where his daughter and the patient played most of the day and the following day The weather was warm and calm and there was constantly a strong smell of solvent on the terrace On May 17 the patient complained of headache and his mother noted swelling of his ankles and face During the preceding week he had a slight upper espiratory tract infection The urine was examined 3 days er by the school nurse who found proteinuria The elling subsided after a few days On June 3 an examina ion at hospital showed signs of acute glomerulonephritis

Histories of 4 patients who reported long exposure to organic solvents before the onset of glomerulo nephritis

Case 7

This 51 year old man had worked as a spray painter since 1939 Between 1944 and 1954 his full time occupation con sisted of spraying lacquer without wearing a mask but after that he painted only a few hours a week At the end of July 1974 he developed fever and a sore throat Two weeks later he developed symptoms and signs of acute glomerulonephritis and nephrotic syndrome

Case 10

This 36-year-old male engineer inspected new blocks of flats in construction For 3 months every year since 1967 he spent 2–3 hours a day in rooms in which the air was heavily contaminated with vapours from paints and carpet glue On such days he suffered from a pulsating headache Proteinuria was discovered at medical check ups in 1972 and 1973 In late Dec 1974 his two children had scarlet fever and he himself a sore throat Penicillin treatment was started after 3 or 4 days Nine or 10 days later he developed severe headache and malaise his face and legs swelled and he gained several kg in weight He recovered spontaneously within 4 or 5 days Proteinuria hematuria uremia and hypertension was discovered acci dentally in Oct 1975

Case 8

This 55 year old male had worked all his life in a boat furniture factory After being lacquered the furniture was heat dried The patient prepared the furniture which came directly from the drying chamber and was still warm He suffered constantly from eczema of the hands caused by the paints In early May 1975 he had a severe infection of the eczematous hand After a week he con sulted a doctor who ordered doxycycline A few days later he fell ill with symptoms and signs of acute glomerulonephritis

Case 4

This 12 year old boy had mild congenital ichtyosis Since 1974 he had spent several hours a week assembling plastic aeroplanes He used a glue solved in trichloroethylene In addition he often rode in the van with his father who transported large vessels of glue The boy felt dizzy on several occasions because of the smell from the glue During that period he often suffered from frequency and dysuria but several urine cultures were negative He had a sore throat with fever in early April 1976 About two weeks later symptoms and signs of acute glomerulo-nephritis appeared

DISCUSSION

Most people on this earth have on some occasion been exposed to vapours of organic solvents or

fuels The exposure of most of the present patients did not exceed what most adults have experienced once or several times in their lives Caution must therefore evidently be exercised when drawing any conclusions about the role played by organic solvents in the pathogenesis of acute post streptococcal glomerulonephritis based on the present case histories

However the close relationship between the time of exposure and the acute onset of nephritis is intriguing especially in the 6 patients who had been exposed not more than insignificantly earlier in life Some of the patients may have been exposed earlier but forgotten this since the exposure was not followed by a severe acute illness But such episodes must have been rare since the patients could not recall any in spite of thorough questioning

The chance that an extremely rare event in life such as poststreptococcal glomerulonephritis should follow so closely upon another infrequent event in 6 out of 15 patients without any connection between the two events seems more than unlikely

Further it appears improbable that exposure of as many as 10 out of 15 patients at the time of the streptococcal infection reflects the degree of exposure in the whole population The low incidence of exposure to solvents in the individuals who were infected with nephritogenic streptococci but who did not develop glomerulonephritis argues against this assumption

In this connexion one should recall that the number of patients exposed is probably minimal Four of the five patients in whom exposure was unknown were small children attending a nursery and the interview was held 6 months to 3 years after recovery from the glomerulonephritis Exposure may have occurred without the parents knowing or it may have been forgotten by them

The third finding suggesting a causal relationship between exposure to organic solvents and glomerulonephritis was the result of the review Of 4 patients who had been exposed to solvents after the acute disease the GFR was remarkably low in 2 and moderate in one whereas in 11 patients who had not been exposed or who had ceased to be exposed the fall if any in GFR was at most insignificant Proteinuria and hypertension were noted in only 2 patients Both of them continued to be exposed

Most authors ascribe acute poststreptococcal glomerulonephritis mainly to the consequences of deposition of streptococcal immune complexes in the glomeruli But many disturbing clinical and experimental observations some of which are mentioned below still await an explanation

1) In some cases meeting with all criteria for poststreptococcal glomerulonephritis only C3 was deposited in the glomeruli or IgG and C3 were deposited in a linear—not a granular—fashion as would be expected in a disease caused by immune complex deposition (5, 6)

2) Streptococcal antigen has been demonstrated in the kidney but then in or between the mesangial cells together with fibrin and not on the GBM in connexion with the deposits of IgG and C3 (1 15 19)

3) Attempts to induce a disease identical with human poststreptococcal glomerulonephritis in mice (8 11 23) rats (14) rabbits (16) and baboons (24) have been unsuccessful In the few experiments in which a morphological picture resembling the human diseases has been elicited (2 9) no evidence was produced to prove that the damage was caused by immunological events

4) In epidemics of streptococcal infections caused by nephritogenic strains only a few individuals had clinical symptoms of acute glomerulonephritis (6 7 10 17)

It has been suggested that the presence of circulating streptococcal metabolic products toxic to the GBM may explain some of the conflicting discrepancies mentioned (6 17) Exposure to exogenous nephrotoxic substances such as organic solvents offers another attractive explanation for many of the findings that are inconsistent with the simple theory of a renal immune complex deposition

Most organic solvents have been found toxic to the mammalian kidney (4) Toxic damage to the GBM and/or intoxication of the mesangial or tubular cell function may be responsible for vulnerability of the kidneys against antigen antibodies complement and immune complexes This mechanism may explain why most people infected with nephritogenic streptococci namely those not exposed to solvents or other toxins clear their kidneys from circulating macromolecules and thereby escape the risk of acute glomerulonephritis The progress to renal failure which for unknown reasons is seen in some patients mainly adults may be caused by continuous occupational exposure to organic solvents

ACKNOWLEDGEMENT

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Combination of Fibromuscular Hyperplasia, Renal Aplasia, Hypoplasia or Dysplasia and Otosclerosis Occurring in the Same Individual or the Same Family

Lars Stavenow Betty Henriques Mats Ekberg
Sven Erik Bergentz and Bertil Hood

From the Departments of Medicine I and Surgery University of Lund Malmo General Hospital Malmo and the Department of Medicine Pitea Hospital Pitea Sweden

ABSTRACT We have found 3 reports of fibromuscular hyperplasia (FMH) of the renal arteries with hypertension occurring in 2 siblings as well as a few instances of renal agenesis and unilateral renal aplasia occurring in the same family In this paper we report on FMH of the renal arteries in 2 women one giving birth to a child with renal agenesis, the other to a child with a focally dysplastic hypoplasia of the kidneys A third family is reported, heavily loaded with hypertension and otosclerosis, in which 2 siblings with FMH and otosclerosis were found Another 4 patients without known heredity for hypertension, but with FMH combined with renal or urinary tract anomalies are also reported The findings are discussed, particularly in relation to the findings in a large material of chronic non obstructive pyelonephritis, where in those with well maintained renal function, hypertension below the age of 40 was found predominantly in females with a positive family history of hypertension and signs highly suggestive of infected hypoplasia or dysplasia of the kidney

In a longitudinal study of a large material of chronic non obstructive pyelonephritis Bengtsson et al (1) found that 6 patients in the younger age groups (20–40 years) had hypertension at normal or only slightly impaired renal function All of these patients had infected hypoplasia or dysplasia of the kidney and 5 of them had a positive family history of hypertension

Does the hypoplastic or focally dysplastic kidney only unmask a genetic disposition for hypertension? Or is there a tendency in some hypertensive patients with hypoplasia or dysplasia of the kidney to be associated with renal or other malformations within the same family? We have found no systematic study of this in the literature but a few scattered reports of isolated instances (4 9 11 18)

We have observed 3 families heavily loaded with hypertension in which fibromuscular hyperplasia (FMH) and/or renal or other malformations occurred in several of the family members We will also report on 6 patients with FMH and renal or urinary tract anomalies 4 of whom had hypertension

CASE HISTORIES

Data on the 12 subjects described below are summarized in Table I

Family I

Case Ia Female born in 1938 No known family history of hypertension or kidney disease Rubella measles and parotitis in childhood In 1962 a BP of 160/110 was registered towards the end of her first pregnancy No proteinuria or infection No abnormality has so far been noticed in this child Spontaneous abortion occurred during the third month of her second pregnancy in 1964 In that year her BP was 220/125 Renal aortography showed changes typical of FMH at the distal end of the right renal artery and extending into the upper main branch for 1 5 cm Right kidney inulin clearance (C_{in}) 37 PAH clearance (C_{PAH}) 194 ml/min left C_{in} 93 C_{PAH} 505 ml/min There was no distinctive stenotic pattern in inulin U/P sodium water potassium phosphate or osmole excretion Reconstructive vascular surgery was considered technically difficult and the BP was well controlled on chlorthalidone betanidine and hydralazine This regimen was continued and maintained during a third pregnancy in 1967 terminating 6 weeks before full term with a still born child (case 1b)

Case 1b Premature male infant dying in respiratory distress syndrome after 15 hours Autopsy showed hyaline membranes in the lungs severe athelectasis (hypoplasia of the lungs?) congenital heart anomaly (most like Fallot s tetralogy) Complete absence of the kidneys ureters and bladder A catheter passed through the urethra went directly into the urachus

Table I *Clinical radiological and functional features of the 12 subjects*

	Ia	Ib	IIa	IIb	IIc	IIIa	IIIb
Sex	♀	♂	♀	♀	♀	♀	♂
Age at time of diagnosis (y)	26	–	49	12	21	39	47
FMH	Dextral		Bilateral		Bilateral	Bilateral	Bilateral
Malformations of urinary tract*		Complete absence of kidneys ureters and bladder	Left bifid renal pelvis left focal dysplasia	Bilateral renal hypoplasia	Double ureter and pelves bilaterally		
C_{In} (ml/min)							
R	37		40	67 (total)	128 (total)	54	52
L	93		29			64	69
C_{PAH}							
R	194		128	467 (total)		230	261
L	505		91			265	312
BP at time of diagnosis (mmHg)	220/125		230/120	200/160	150/100	280/150	220/130
Treatment	Medical		Medical	Medical		Bilateral arterenal reconstruction with venous graft	Medical
Associated malformations		Pulmonary hypoplasia congenital heart anomaly				Otosclerosis	Bilateral conduction defect

Family II

Case IIa Female born in 1919 Her father and mother both died from myocardial infarction at the age of 74 the mother after 15 years of hypertension The patient had normal BP in 1955 but in 1968 at the age of 49 her BP was 230/120 Investigation disclosed a left bifid renal pelvis The upper pelvis consisted only of an elongated narrow recess From the lower pelvis one calyx approached close to the margin of the kidney The margin of this kidney showed indentations Both renal arteries showed changes typical of FMH No signs of urinary tract infection either in the history or at examination (Fig 1a) Right kidney C_{In} 40 C_{PAH} 128 ml/min left kidney C_{In} 29 C_{PAH} 91 ml/min Inulin U/P right 142 left 164 Osmole/kg H_2O right 672, left 740 BP easily controlled on drugs

Case IIb Daughter of case IIa born in 1943 Urinary tract infection and hypertension (200/160) discovered in 1955 at the age of 12 I v pyelography showed a small left kidney Papillary impressions were missing. Several calices reached to within a few mm of the lateral kidney margin Right kidney showed similar changes but several normal papillary impressions were found in the upper pole (Fig 1b) Renal aortogram showed normal renal arteries BP control and general health excellent on pentholinium hydralazine and a small dose of reserpine for 15 years In 1969 C_{In} 67 C_{PAH} 467 ml/min moderate pyuria Coli 500000/ml urine Pitressin tannate test 613 mosmol/kg H_2O

Case IIc Daughter of case IIa born in 1946 Towards the end of her two pregnancies in 1966 and 1968 moderate proteinuria and BP 190–210/100–130 At this time there were no signs of urinary tract infection Children born at full term pregnancies appear entirely healthy Admitted twice for study in the Department of Medicine in 1967 and 1969 BP 150–140/100–80 in 1969 Pyuria and coliuria 10 mill /ml C_{In} 128 ml/min Normal concentration capacity I v pyelography showed double ureters and pelves bilaterally Abdominal aortography without remarks At follow-up examinations BP was 150–160 80–90 without antihypertensive drugs No bacteriuria or pyuria.

In 1971 she fell ill with profuse sweatings headache and a BP of 260/190 Examination revealed high levels of cathecholamines and methoxycathecholamines renal arteriography disclosed a tumour at the upper pole of the left kidney Surgical exploration revealed a phaeochromocytoma 5×5 cm histological examination showing no suspicion of malignancy Still in 1978 she feels well and has a normal BP

IV	V	VI	VII	VIII
♀	♀	♀	♀	♀
19	27	37	59	45
Dextral	Dextral	Dextral	Dextral	Dextral
Ectopic ureter sin double pelves and ureter sin hydronephrosis sin	Left renal hypoplasia	Bilateral renal hypoplasia	Left renal hypoplasia	Bilateral renal dysplasia
60 (total postop)				
200/110	180/120	160/90	150/85	130/80
Medical + right renal artery reconstruction	Medical	Medical + bypass operation with venous graft		
		Pectus excavatum		

Family III

Case IIIa Female born in 1929 8th of 9 siblings Her 72 year-old father has high BP and claudication Her mother died at 54 from a stroke and was known to have had systolic BPs of 275 and above The mother had and 2 sisters have otosclerosis Three sisters operated on for thyreotoxicosis One brother (III*b*) and one sister have hypertension Two operations for otosclerosis at the age of 27 and 28 Recurrent cystitis at the age of 16 Acute pyelonephritis elevated BP and proteinuria during pregnancy in 1952 Proteinuria reappeared during the following 3 pregnancies Children—16 10 9 and 6 years—all apparently healthy

In 1968 the patient became tired and breathless and had a BP of 200/160 Eye grounds grade III The renal aortogram disclosed bilateral changes typical of FMH (Fig 2*a*) Right kidney C_{In} 54 C_{PAH} 230 left kidney C_{In} 64 C_{PAH} 265 No definite side differences as regards osmolality inulin U/P or sodium excretion or radiorenograms Bilateral reconstruction with free grafts of the great saphenous vein

Case IIIb Born in 1921 brother of case III*a* Somewhat impaired hearing due to bilateral conduction defect BP 220/130 Renal aortogram showed changes typical FMH both in the right renal artery and in one of t arteries to the left kidney (Fig 2*b*) Right kidney C_{In} 5 C_{PAH} 261 left kidney C_{In} 69 C_{PAH} 312 No definite si difference as regards inulin U/P osmolality or sodiu excretion BP was controlled with a combination propranolol betanidine and hydralazine

Individual subjects

Case IV Female born in 1928 Because of recurre pyelonephritis and hypertension i v pyelography w performed in 1947 and disclosed double renal pelvis ar ureters on the left side Operated on because the le ureter emerged distally to the urinary bladder Persiste hypertension since her last pregnancy in 1955 A left side heminephrectomy was carried out in 1955 because hydronephrosis in the upper renal pelvis The hyperte sion persisted and led to nephrectomy of the left kidney 1956 without effect on her high BP

Further examination revealed a *right* renal arte stenosis due to FMH BP 200/110 was difficult to reg late by medical treatment only and therefore an operatio was carried out in 1966 The stenotic part was resecte and replaced by venous graft. Renal biopsy showed slight arterial hyalinosis Right kidney C_{In} postoperative 60–65 Her BP has been satisfactorily controlled sinc then with α methyldopa betanidine spironolactone ar chlorthalidone

Case V Female born in 1945 Headache for sever years Three normal deliveries one abortion In 1972 B 180/120 Renal angiography showed a renal arte stenosis appearing like FMH with a slight poststenot dilation The right kidney was of normal size the le kidney was small 4 2×9 3 cm deformed in its uppe portion and estimated to perform 20% of the total ren function The artery of this kidney was somewhat narro but otherwise normal Regional blood flow examinatio with gamma camera spoke against a renal artery stenos of functional significance The total renal function wa normal Good BP control on propranolol

Case VI Female born in 1937 Her grandmother ha hypertension The patient went through 3 pregnancies i 1956 1957 and 1963 all complicated by hypertension an miscarriage in mens VI–VII Antihypertensive treatme since 1956 The difficulty in regulating BP by medic treatment alone and the appearance of hypopotassem resulted in a detailed investigation Pectus excavatum wa noted at the physical examination Renal angiograph showed considerable fibromuscular stenosis in a ventr artery on the right side with considerable ren *parenchymal hypotrophy of the part of the kidney su* plied by this branch The parenchyma in the rest of th right kidney and in the whole of the left kidney was als much reduced BP was normalized with large doses c clonidine chlorthalidone hydralazine and spiron lactone

Case VII Female born in 1918 BP 150/85 Because c urinary tract infections i v pyelography was performe revealing a small left kidney 7×3 cm and a normal righ kidney Renal arteriography showed a uniform narrowin of the left renal artery of 3 mm size and the kidne parenchyma was heavily reduced A moderate FMH wa

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individual in a thousand The condition is encountered most frequently in the still born or newborn because lethal malformations are often present in other organ systems—congenital heart disease myelomeningocele etc Bound (2) reported two cases of congenital absence of one kidney an 8 year old boy and his maternal uncle Gorvoy et al (6) found unilateral renal agenesis in 2 brothers and failed to find any abnormalities in pyelograms of the mother father and a 10-year old sibling

Bilateral agenesis seems more rare (4) It is often associated with other gross defects and has only twice been reported to occur in the same family in the second and fourth child (11) Schmidt et al (18) reported bilateral aplasia in 2 sisters the mother being apparently healthy The mothers in Davidson s and Ross series (4) were reported to be healthy Peschke (15) reported a case whose mother had congenital pulmonary stenosis and a sister hydronephrosis Hilson s case 3 (9) was born without kidneys A cousin had no left kidney and a grandmother had some kidney trouble

The male sex preponderance in bilateral renal agenesis is stated to be 70–80% again contrary to the figures found in hypoplasia/dysplasia

In a survey of 180 congenital tract abnormalities found in 2 153 consecutive autopsies of infants and children up to 12 years of age Rubenstein et al (16) found simple hypoplasia to be infrequently associated with other anomalies of the urinary tract but malformations of other organs were present in 32 of 58 cases In dysplastic hypoplasia urinary tract malformations were frequent abnormalities of other organs infrequent It is hardly conceivable that the association of FMH in the same family in one case with bilateral agenesis in another with dysplastic hypoplasia and in the third with another case of FMH is simply a chance occurrence The same applies to the occurrence of FMH and renal or urinary tract malformations in the same patient in 5 cases That total renal agenesis is far more prevalent in males whereas FMH as well as hypoplasia/focal dysplasia occur much more frequently in women makes the matter more intriguing

There was a heavy association of FMH with otosclerosis appearing early in life in family III One of us (B H) has while serving in another centre observed at least two more subjects with FMH on one side and no contralateral kidney as well as one subject with FMH and a heavy occurrence of otosclerosis and hypertension in the family We have not been able to relocate the records of these subjects We have found no such reports earlier in the literature supporting this association

The appearance of pectus excavatum in our case VI is of interest This anomaly occurs frequently in Marfan s syndrome the well known congenital and hereditary disorder of connective tissue (12) and has been reported in combination with FMH of carotid arteries as well as an intracranial aneurysm in the same patient (17)

All these findings suggest that FMH at least in certain cases may be a reflection of a hereditary mesenchymal disorder rather than a specific arterial defect

It is concluded that it may be fruitful to screen the relatives of subjects with diagnosed renal malformations as well as those with diagnosed FMH for hypertension If hypertension is found this ought to be carefully followed up

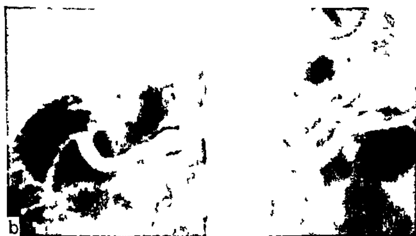


Fig 2 (a and b) Siblings with changes typical of fibromuscular hyperplasia of renal arteries I v pyelogram entirely normal (*a*) Case III*a* (*b*) case III*b*

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Urinary Excretion of Basement Membrane Antigen in Normal Persons and Patients with Febrile Proteinuria —Quantitated by Means of Rocket Immunoelectrophoresis

Kirsten Henriksen and Herluf Jensen

From the University Clinic for Infectious Diseases and Medical Department III Municipal Hospital Copenhagen Denmark

ABSTRACT The urinary excretion of a glomerular basement membrane like antigen was quantitated in 19 adult normal subjects and in 15 patients with febrile proteinuria by means of rocket immunoelectrophoresis In the normal persons the excretion averaged 59 (S D 8 9) U/24 hours' creatinine clearance An increased excretion was demonstrated in 80% of the patients There was a significant relation between the urinary excretions of albumin and protein in the patients with febrile proteinuria The basement membrane antigen was also demonstrated in normal human serum, liver and placenta No unusual basement membrane antigen could be demonstrated in the urine from patients with febrile proteinuria

Soluble glomerular basement membrane (GBM) like antigens are excreted in normal human urine (NHU) (8 14 16) This urinary basement membrane (BM) material is a glycoprotein (16) which shares antigenic determinants with BM of other organs and it has also been demonstrated in normal human serum (NHS) (16) The existence of proteinuria in non renal infections has been clearly documented (3 7 9 15 22) though the mechanism is still unknown

Since a change in the amount—as well as in the nature—of the urinary BM excretion may prove to be a better indicator of renal disease than the excretion of other proteins the purpose of the present investigation has been to quantitate this excretion in NHU and compare the excretion to the urinary excretion in patients with febrile diseases

STUDY POPULATION

The normal group comprised 19 adult persons 11 females and 8 males aged 25–44 years all without any history of kidney disease Their endogenous creatinine clearances (Cl_{cr}) ranged from 1 3 to 2 4 ml/sec (Table I)

The patient group consisted of 15 patients with febrile illnesses admitted to the emergency section of the University Clinic for Infectious Diseases Copenhagen 5 females and 10 males aged 15–73 years Their rectal temperature on admission was >38 C The patients had no previous or present kidney diseases and they had normal urine sediment by microscopy and no growth of bacteria in the urine Immediately after admission material from the infected organ was obtained for microscopy and cultivation The details are listed in Table II

METHODS

Twenty four hour portions of urine were quantitatively collected and sodium azide was added as preservative to give a final concentration of 0 1% (w/v) The patients urines were collected for 24 hours from 12 p m after admission The urines were concentrated by means of low pressure ultrafiltration using either Visking dialysis tubes 23/32 inches (4) or Minicon B chambers purchased from Dansk mikrobiologisk A/S One 24-hour urine was collected from 17 normal persons two portions from one person and three from one person Protein excretion was determined by a biuret method slightly modified after Savory et al (17)

Antigens and antisera

Human GBM (HGBM) was isolated as described by Spiro (18) and modified by Westberg and Michael (23) Human liver and placenta were prepared as earlier described (8) HGBM liver and placenta were solubilized with collagenase obtained from Worthington Biochemical Corp New Jersey Antisera to HGBM (anti HGBM) were prepared as earlier described (8) and absorbed with lyophilized NHS

Abbreviations BM=basement membrane GBM=glomerular basement membrane HGBM=human glomerular basement membrane anti HGBM=antiserum to HGBM NHU=normal human urine NHS=normal human serum U=unit(s) Cl_{cr}=creatinine clearance

Table I Urinary excretion of protein albumin and BM antigen in 19 normal persons

Subj no	Sex	Age (y)	Protein (mg/24 h)	Albumin (mg/24 h)	Cl_{cr} (ml/sec)	BM antigen U/24 h	BM antigen U/24 h/Cl_{cr}
1	♀	36	96	5 67	1 77	117	66
2	♂	40	70	3 30	1 54	90	58
3	♂	41	99	4 70	1 81	114	63
4	♂	39	99	4 80	1 78	94	53
5	♂	29	75	4 25	1 71	106	62
6	♀	29	42	5 37	1 23	78	63
7	♀	43	59	4 68	2 08	100	48
(	♀	37	47	5 40	1 82	118	65
1	♀	37	107	5 30	1 70	119	70
8	♀	29	108	4 93	1 30	102	78
9	♀	34	103	2 88	1 40	90	64
10	♀	32	82	4 95	1 43	78	55
11	♂	28	51	7 84	1 88	125	66
12	♂	34	84	5 91	1 76	102	58
7	♀	44	42	3 26	1 69	90	53
13	♀	35	48	10 66	2 38	122	51
14	o	28	47	5 28	2.25	128	57
15	♀	39	47	3 47	2 01	106	53
16	♂	43	52	4 90	1 77	134	76
17	♀	29	56	5 64	1 81	92	51
18	♀	25	30	4 20	1 41	66	47
19	♀	44	50	5 67	1 75	80	46

Electrophoretic techniques

...ed immunoelectrophoresis was carried out as de... earlier (19 21) using 1% agarose gel (thickness 1 ... in barbital buffer pH 8 6 ionic strength 0 02 The ...t-dimension electrophoreses of collagenase-digested HGBM liver and placenta concentrated NHU and concentrated NHS were run at 10°C applying 10 V/cm for 1 hour The second-dimension electrophoreses were run at 10°C applying 3 V/cm for 20 hours Tandem-crossed immunoelectrophoresis was performed as described by Kroll (11)

Albumin in concentrated and unconcentrated urine was quantitated by means of immunoelectrophoresis in antibody-containing gel (12 20) Rabbit antihuman albumin was obtained from Dakopatts A/S Copenhagen These values were used to estimate the degree of concentration of the urines The rocket immunoelectrophoresis was further employed to quantitate the HGBM like antigen found in the urine the agarose gel containing absorbed anti HGBM A randomly chosen concentrated NHU was employed as reference in 4 different dilutions The 24-hour excretion of GBM like material in this urine was defined as 100 U and the excretion per 24 hours of GBM like material in urines was calculated accordingly

In order to evaluate the type of proteinuria agarose gel electrophoresis was performed in 0 075 M veronal buffer pH 8 4 containing 1% (w/v) agarose The electrophoresis was run for 45–60 min at 20 V/cm

Chromatography

Concanavalin A covalently bound to Sepharose 4B by the cyanogen bromide method was obtained from Pharmacia Fine Chemicals AB as a suspension of 100 ml sedimented gel in acetate buffer solution (0 1 M pH 6) containing 1 M sodium chloride 1 mM calcium chloride and 0 02% merthiolate (Con A Sepharose) NHS dialysed against the starting buffer was introduced at a speed of 15 ml/hour into the bed (28×2 cm) of Con A Sepharose After washing with 400 ml of the starting buffer displacement was effected with 300 ml phosphate buffer (0 1 M sodium phosphate 1 M sodium chloride (pH 7 2)) followed by elution with 300 ml 0 1 M borate buffer (pH 6) (10) The two eluates were concentrated separately by low pressure ultrafiltration The temperature during the procedures was 4 C The same procedure was employed for pooled concentrated NHU

RESULTS

Normal persons

The protein excretion ranged from 30 to 108 mg/24 hours (Table I)

Crossed immunoelectrophoresis with concentrated NHU as antigen and anti HGBM as antibody demonstrated one precipitate with α mobility This precipitate cross reacted in tandem crossed immunoelectrophoresis with one of the precipitates formed between HGBM and anti HGBM and also with one of the precipitates formed between placenta and liver and anti HGBM as previously described (8)

No further precipitate was demonstrated after concentrated NHU had been subjected to

Table II *Urinary excretion of BM antigen protein and albumin and agarose gel pattern of the proteinuria in 15 patients with febrile illnesses*

NI=not identified T=tubular G=glomerular GT=glomerulotubular F=broad α_1 fast α_2

Pat. no	Sex	Age (y)	Diagnosis	Infecting micro-organism	Cl_{cr} (ml/sec)	BM antigen U/24 h	BM antigen U/24 h/ Cl_{cr}	Protein (mg/24 h)	Albumin (mg/24 h)	Agarose gel pattern
1	♀	43	Gastroenteritis ac	Salmonella typhimurium	1 1	95	86	486	23	T
2	♂	42	Mumps		1 6	205	126	1 690	276	GT
3	♂	15	Fever of unknown origin		2 6	147	95	401	17	F
4	♂	15	Pneumonia	Pneumococcus	1 8	116	83	2 283	196	T
5	♂	60	Meningitis	Pneumococcus	1 1	305	277	2 180	413	GT
6	♂	44	Gastroenteritis	NI	1 4	219	156	1 212	98	T
7	♂	16	Pneumonia	NI	0 7	103	141	490	93	G
8	♀	62	Meningitis	Pneumococcus	1 4	103	74	694	100	F
9	♂	33	Post vacc encephalitis		1 5	124	83	1 333	288	G
10	♂	17	Meningitis	Neisseria meningitidis	2 5	131	52	784	121	T
11	♀	25	Lymph meningitis	NI	1 2	108	72	426	142	G
12	♂	26	Mumps		1 8	162	90	1 360	209	G
13	♀	73	Pneumonia	NI	1 7	170	101	1 010	179	GT
14	♀	17	Pneumonia	Pneumococcus	1 4	246	178	1 910	411	GT
15	♂	22	Meningitis	Neisseria meningitidis	2 8	297	108	2 687	300	T

chromatography Crossed immunoelectrophoresis with concanavalin A chromatographed NHS as antigen and anti HGBM as antibody demonstrated one precipitate which—in tandem crossed immunoelectrophoresis—with concentrated NHU as the other antigen showed identity with the BM like antigen in NHU

Rocket immunoelectrophoresis The albumin excretion ranged from 2 8 to 10 6 mg/24 hours i e within normal limits (1) (Table I) The excretion of GBM like material was 66–134 (mean 102 S D 18 0) U/24 hours and 46–78 (mean 59 S D 8 9) U/24 hours/Cl_{cr} One person s (subj 1) urine was investigated three times and the respective excretions per 24 hours were 117 118 and 119 U Another person s (subj 7) excretion was investigated twice and was 100 and 90 U/24 hours respectively

The frequency distribution of BM antigen excretion expressed as U/24 hours as well as U/24 hours/Cl_c is depicted in Fig 1

Patients

The 24 hour excretion of protein ranged from 401 to 2687 mg and of albumin from 17 to 413 mg The agarose patterns were all abnormal according to Laurell (13) 4 glomerular 4 glomerulotubular 5 tubular and 2 febrile with broad α_1 and fast α_2 (Table II)

The urinary excretion of BM like material was 95–305 U/24 hours The excretion of BM like material/24 hours/Cl_{cr} was 52–277 U (Table II) Compared with the normals the excretion of BM like material was elevated in 8 patients and the BM excretion/24 hours/Cl_c was elevated in 12 patients In one urine (pat 4) the BM excretion was only slightly elevated but the excretion of protein was considerable (2283 mg) the excretion of albumin was comparatively low and the agarose gel showed a tubular pattern

However in 2 urines (pats 6 and 15) with tubular pattern high protein excretion and comparatively

Table I *Urinary excretion of protein albumin and BM antigen in 19 normal persons*

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On Controversial and Open Questions about the Course and Complications of Non-Obstructive Urinary Tract Infection in Adult Women

Follow up for up to 80 Months of 707 Participants in a Population Study and Evaluation of a Clinical Series of 36 Selected Women with a History of Urinary Tract Infection for up to 40 Years

Nils Alwall

From the Health Service of Malmohus County District University Hospital Lund Sweden

ABSTRACT In an earlier report of the results of the initial medical examination in our population study from the period 1969–75 on non-obstructive urinary tract infection (UTI) in non pregnant women it was shown that the negative conclusions published by others on the importance of UTI and the value of early diagnosis rested on insecure grounds, owing partly to the test used in determination of renal function and partly to the selection of the material for the population studies After, in most cases, long term treatment of 142 bacteriuric women there was significant improvement of the defective maximum urinary concentration ability, which continued until the end of the follow up period There was no significant difference between the subgroups of women who had had and had not had symptomatic UTI in the past 659 women were followed up for 36–80 months They were divided into the same series as those to which they belonged initially in the earlier study 1) 212 controls with no past history of subjective symptoms of upper or lower UTI and no urinary abnormalities i e neither bacteriuria nor pyuria at the time of the first medical examination 2) 180 women with a past history of symptomatic UTI but no urinary abnormalities 3) 173 women with sterile pyuria, and 4) 94 women with bacteriuria/pyuria About 40 % of the women in the latter two series had no past history of UTI In comparison with the other series series 1 had a significantly lower number of women with symptomatic UTI and newly diagnosed hypertension during the follow up period as well as of women with bacteriuria and/or pyuria at the final examination Between series 2 3 and 4 there were significant differences mainly with respect to the final finding of pyuria the number of such cases was highest in series 3 The importance of symptomatic UTI as a criterion is limited by the overlapping that is represented by the women in series 3 and 4 who had no past history of such symptoms at the time of the initial medical examination Systematic radiological re-examinations could not be carried out because of inadequate resources. The controversial question of a possible relation between non-obstructive UTI and progressive renal damage in normotensive adult women is illustrated by the development of bilateral papillary necrosis and/or shrinking of the kidneys during a 6 1 (1–15) years interval following the finding of a normal i v urogram in 33 selected patients aged 49 0 (26–71) who had a past history of UTI for 14 7 (2–40) years Just over two thirds of them had had episodes of acute pyelonephritis In two additional cases i v urography showed no abnormalities but renal angiography revealed parenchymal damage with scars. Furthermore biopsy showed evidence of chronic pyelonephritis with an acute inflammatory process in a patient with normal urogram and normal angiogram The reliability of radiographic methods and the possibility of demonstrating progressive kidney damage by i v urography in population studies with a relatively short follow up period are discussed To what extent patients with UTI run the risk of progressive kidney damage of clinical importance still seems to be an open question

Our results from the initial examinations of non pregnant women and males aged 21–70 over the period Jan 1969 – Sept 1975 have been reported (3) Recently published surveys of population studies with follow up for a maximum of 6 or 7 years led to the conclusion that such studies are not justifiable since uncomplicated urinary tract infection

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Paper read in part at the International Workshop on Urinary Tract Infection in Rostock May 4 1975 organized by the Society of Nephrology of the G D R. and sponsored by the International Society of Nephrology and at the Meeting of the Hellenic Society of Nephrology (D J Valtis Memorial Lecture) in Thessaloniki March 31 1978

Author's address Torsv 14 S-223 56 Lund Sweden

(UTI) would not cause morbidity or progressive renal damage in normotensive non pregnant adult women (6, 7) The results were widely accepted without criticism In consequence our resources were gradually cut down and came to an end in Oct 1975 Medical and radiographic examinations could be repeated on only a limited scale during the follow up

The present paper is a report of a follow up of 707 women for up to 80 months It covers among other features the presence of subjective symptoms of upper and/or lower UTI the occurrence of new cases of hypertension and the results of a final medical examination at the end of the observation period The resources were not sufficient to allow systematic radiological follow up

The development of abnormalities of pyelonephritis type after previously normal urograms was studied in a selected clinical series of 36 adult women with a history of UTI for up to 40 years The reliability of i v urography for diagnosing pyelonephritis is illustrated by two selected cases with normal urograms in whom pyelonephritis was diagnosed by renal angiogra hy These diagnostic problems which perhaps have not been considered sufficiently in lished reports of population studies are further s rated by the biopsy findings in a woman who acute exacerbations of a chronic pyelonephritis and no urographic or angiographic abnormalities

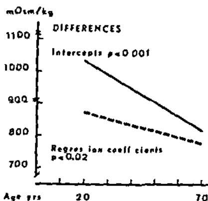


Fig 1 Improvement of the defective maximal concentrating ability (– – –) in 142 non pregnant adult women following elimination of bacteriuria (——)

METHODS AND STUDY POPULATION

The methods and definitions used were the same as those reported earlier (3) The study population comprised 707 women who took part in a population study (*a* and *b*) and a selected clinical series of 36 women (*c*)

(*a*) 142 adult non pregnant women in whom bacteriuria was diagnosed at the initial medical examination Maximal urinary concentration ability was examined after eradication of the bacteriuria by in most cases long term treatment The urine was sterile after treatment for 3–4 weeks in 22% of the cases most of them belonging to the two lowest age groups (21–30 and 31–40 years) 43% of all the cases were treated for more than 6 months A sulpha drug in the last few years Bactrim® was in most cases used initially unless the result of the bacteriological sensitivity test indicated the need for another preparation Nitrofurantoin (Furadantin®) was used during some periods in long term treatment The use of antibiotics was restricted as far as possible The following bacterial species were found E coli in 79 4% E coli and coliform rods in 7 8% coliform rods in 7 8% Proteus sp in 1 9% and others in 3 1% The distribution agrees with that noted in a previously published part of the bacteriuric series (2) serotyping was not performed

(*b*) 659 adult women 94 of whom are included in those 142 mentioned above (under *a*) The follow up for at least 36 months included the course and a final examination Irrespective of the results the individuals remained in the series to which they had belonged in the initial medical examination (3)

Series 1 212 women with no past history of upper and/or lower UTI before the initial medical examination normotensive and with no urinary abnormalities i e neither bacteriuria nor pyuria The mean age was 48 4 years

Series 2 180 women with no urinary abnormalities who had a past history of UTI mean age 48 7 years The present series comprises ten new cases

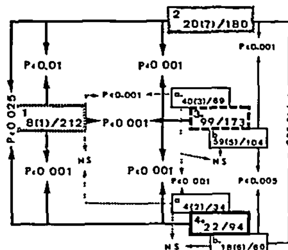
Series 3 173 women with sterile pyuria (104 (60 1%) with and 69 (39 9%) without past history of UTI) Mean age in the total series was 52 3 years and in the two subgroups *a* and *b* 51 8 and 53 2 years respectively

Series 4 94 women with bacteriuria/pyuria (60 (63 8%) with and 34 (36 2%) without past history of UTI) Mean age in the total series 47 7 years and in the two subgroups 47 0 and 48 4 years respectively At the initial examination 60% of the women in the latter two series had a past history of UTI (3)

Because of insufficient resources the number of women in series 1 at this follow up was reduced to 65 4% of the original material so as to make more room for the other series This probably did not influence the results since the subjects were called successively from different areas of our district The present series 2 3 and 4 comprise 93 7% of the original number of individuals (3)

The length of the follow up in the four series exceeded 3 years in 96 2 (95 5–97 5) % 4 years in 92 1 (87 3–95 0) % 5 years in 69 4 (68 2–70 7) % and 6 years in 16 6 (13 6–17 9) % The shortest period was 36 and the longest 80 months

As the resources became inadequate it was impossible to summon the women for medical re-examination at fixed intervals as planned Therefore questionnaires were mailed containing questions referring to among other



in series 1 than in series 2 ($p<0\ 05$) 3 ($p<0\ 02$ and 4 ($p<0\ 001$) respectively

Occurrence of hypertension during follow up in 490 initially normotensive women (Fig 5)

Hypertension was diagnosed during the follow in 2 8% of the controls in series 1 all of whom we normotensive initially In series 2 3 and 4 63 64 7 and 55 3% of the women were normotensive the time of the initial examination during t follow up hypertension was diagnosed in 13 2 18 and 21 2% of these The difference was significa ($p<0\ 001$) only between series 1 and the oth series

Maximal urinary concentrating ability

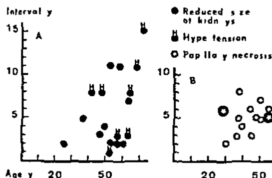


Fig 6 Ages of 31 selected normotensive female patients with UTI at the time when i v urogram first showed abnormalities of the type of chronic pyelonephritis and interval from the radiological examination with normal findings at i v urography (*A*) Findings of contracted kidneys in 18 cases Hypertension (*H*) was diagnosed in 8 cases later in the course than the shrivelling of the kidneys (*B*) Findings of bilateral papillary necrosis in 11 women (small symbols) with reduced size of kidneys (contracted kidneys) and in 2 women (large symbols) with kidneys of normal size

the mean value at the end of the follow up 875 1 mOsm (±110 4 and ±11 5) The difference between the initial and the final mean values was significant ($p<0\ 001$)

33 selected adult female patients with non obstructive chronic UTI and progressive kidney damage

This study covers about 30 years New diagnostic methods were introduced during this period for instance percutaneous kidney biopsy (1 4) and renal angiography It was not until less toxic contrast media became available that large doses could be used for producing i v urograms and that arterial angiograms could be recorded even at greatly impaired renal function Earlier the indications had been limited owing to the risk of complicating acute renal insufficiency which could prove fatal (4 5) This complication also occurred at retrograde pyelography (4) which involves the additional risk of infection Open biopsy was performed in some cases

Normal sized kidneys and normal urograms were noted initially in the 33 women after mostly long lasting UTI Two of them underwent repeated examinations that showed no abnormalities 79% of these examinations were carried out in the 1950s or earlier

Repeated investigation during the subsequent course of the disease showed contraction of both kidneys in 29 women Four underwent a further two examinations and 3 cases were checked by X ray on three occasions In 6 of the latter 7 patients one kidney decreased in size more and a little faster than the other during the continued observation At the last examination the shrivelling of the two kidneys was equal Contraction of one kidney was noted in two cases The longitudinal axis of the kidneys decreased by an average of 2 1 (1 0–5 5) cm

Kidney size remained unchanged in 2 women throughout the observation period but papillary necrosis of both kidneys developed

Fig 6*A* shows the age of 18 women at the time when the shrivelling of the kidneys was first noted the mean age was 53 6 (26–71) years The interval from the radiological examination showing no abnormalities averaged 5 6 (1–15) years These figures do not include the additional period of 5 and 8 years respectively in which the afore mentioned further shrivelling of the kidneys was noted on the abdominal plain film at repeated examinations 16 women stated that they had been troubled with UTI for as an average the last 13 8 (2–40) years the information was uncertain in 2 cases 61% had had episodes of acute pyelonephritis type A diagnosis of papillary necrosis was not attempted in this group as renal function was so impaired that at this time the use of contrast media was considered to be contraindicated The diagnosis of chronic pyelonephritis was verified in 50% at biopsy and/or autopsy Hypertension was diagnosed in 8 cases later in the course than the shrivelling of the kidneys at the mean age of 58 1 (42–71) years

Fig 6*B* shows that the mean age of 11 women was 42 9 (30–55) years when in spite of impaired renal function bilateral shrivelling and papillary necrosis were diagnosed by i v urography in the second half of the 1950s and later on an average 4 9 (2–8) years after the initial examination showing no abnormalities The diagnosis of chronic pyelonephritis was verified by renal angiography in 3 and by biopsy and/or autopsy in 5 cases Uraemia developed during the observation period in 4 patients Three fourths had a past history with episodes of acute pyelonephritis The interval between the onset of UTI and the finding of the afore mentioned radiological abnormalities averaged 16 9 (8–34) years in the 9 cases in which the information was certain

Fig 4 Diagnosis of bacteriuria and/or pyuria (bacteriuria/pyuria) at the final medical examination in series 1 2 3 and 4

occurred in 0 5 81 1 62 1 and 54 6% of the women in series 1 2 3 and 4 respectively These figures include episodes of upper UTI

Bacteriuria and/or pyuria at the final examination in 659 women (Fig 4)

medical examination that completed the period bacteriuria and/or pyuria was agnosed in 3 8 11 1 57 5 and 23 0% respectively of the women in the four series The difference is significant between series 1 and 2 ($p<0\ 01$) series 1 and 3 ($p<0\ 001$) series 1 and 4 ($p<0\ 001$) series 2 and 3 ($p<0\ 001$) series 2 and 4 ($p<0\ 025$) and series 3 and 4 ($p<0\ 001$)

The control series 1 had a significantly lower number of pyurics than the subgroup *a* of series 3 with no past history of UTI ($p<0\ 001$) but there was no significant difference between series 1 and 4*a* Series 2 had a significantly lower number than the corresponding subgroups (*b*) with a past history of UTI of series 3 ($p<0\ 001$) and of series 4 ($p<0\ 005$) The number in the first mentioned subgroup *a* of series 4 was significantly lower than that of series 3 ($p<0\ 001$) the difference between the corresponding numbers in the last mentioned subgroups (*b*) was similar ($p<0\ 005$) The differences between the two subgroups *a* and *b* respectively in series 3 and in series 4 were not significant

The differences in the number of women with bacteriuria were not significant between series 2 3 and 4 whereas the number was significantly lower in series 1 than in series 2 ($p<0\ 05$) 3 ($p<0\ 025$) and 4 ($p<0\ 001$) respectively

Occurrence of hypertension during follow up in 490 initially normotensive women (Fig 5)

Hypertension was diagnosed during the follow up in 2 8% of the controls in series 1 all of whom were normotensive initially In series 2 3 and 4 63 3 64 7 and 55 3% of the women were normotensive at the time of the initial examination during the follow up hypertension was diagnosed in 13 2 18 8 and 21 2% of these The difference was significant ($p<0\ 001$) only between series 1 and the other series

Maximal urinary concentrating ability during follow up

Series 1 The mean value at the initial medical examination was 920 3 mOsm (S D ±82 8 and S E ±7 5) At the final medical examination at the end of the follow up period the mean value was 931 0 mOsm (±88 8 and ±8 0) and in the middle of the period 926 6 (±83 1 and ±7 6) There were no significant differences between these three values

Series 2 The initial mean value of 910 7 mOsm (±80 2 and ±7 9) was significantly higher ($p<0\ 05$) than the value obtained at the end of the follow up which was 873 4 mOsm (±94 4 and ±9 4)

Series 3 The initial mean value of 876 1 mOsm (±104 8 and ±8 0) was significantly lower ($p<0\ 025$) than the final value of 901 4 mOsm (±93 9 and ±7 1)

Series 4 The initial low mean value of 803 9 mOsm (±143 4 and ±15 0) increased significantly ($p<0\ 001$) after eradication of the bacteriuria to 908 7 mOsm (±135 9 and ±14 2) There was no significant difference between the latter value and

Fig 5 Occurrence of new cases with hypertension in initially normotensive women in series 1 2 3 and 4

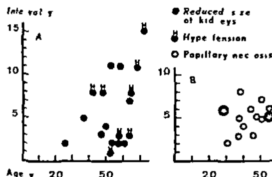


Fig 6 Ages of 31 selected normotensive female patients with UTI at the time when i v urogram first showed abnormalities of the type of "chronic pyelonephritis" and interval from the radiological examination with normal findings at i v urography (*A*) Findings of contracted kidneys in 18 cases Hypertension (*H*) was diagnosed in 8 cases later in the course than the shrivelling of the kidneys (*B*) Findings of bilateral papillary necrosis in 11 women (small symbols) with reduced size of kidneys (contracted kidneys) and in 2 women (large symbols) with kidneys of normal size

the mean value at the end of the follow up 875 1 mOsm (±110 4 and ±11 5) The difference between the initial and the final mean values was significant ($p<0.001$)

33 selected adult female patients with non-obstructive chronic UTI and progressive kidney damage

This study covers about 30 years New diagnostic methods were introduced during this period for instance percutaneous kidney biopsy (1 4) and renal angiography It was not until less toxic contrast media became available that large doses could be used for producing i v urograms and that arterial angiograms could be recorded even at greatly impaired renal function Earlier the indications had been limited owing to the risk of complicating acute renal insufficiency which could prove fatal (4 5) This complication also occurred at retrograde pyelography (4) which involves the additional risk of infection Open biopsy was performed in some cases

Normal sized kidneys and normal urograms were noted initially in the 33 women after mostly long lasting UTI Two of them underwent repeated examinations that showed no abnormalities 79% of these examinations were carried out in the 1950s or earlier

Repeated investigation during the subsequent course of the disease showed contraction of both kidneys in 29 women Four underwent a further two examinations and 3 cases were checked by X ray on three occasions In 6 of the latter 7 patients one kidney decreased in size more and a little faster than the other during the continued observation At the last examination the shrivelling of the two kidneys was equal Contraction of one kidney was noted in two cases The longitudinal axis of the kidneys decreased by an average of 2 1 (1 0–5 5) cm

Kidney size remained unchanged in 2 women throughout the observation period but papillary necrosis of both kidneys developed

Fig 6*A* shows the age of 18 women at the time when the shrivelling of the kidneys was first noted the mean age was 53 6 (26–71) years The interval from the radiological examination showing no abnormalities averaged 5 6 (1–15) years These figures do not include the additional period of 5 and 8 years respectively in which the afore mentioned further shrivelling of the kidneys was noted on the abdominal plain film at repeated examinations 16 women stated that they had been troubled with UTI for as an average the last 13 8 (2–40) years the information was uncertain in 2 cases 61% had had episodes of acute pyelonephritis type A diagnosis of papillary necrosis was not attempted in this group as renal function was so impaired that at this time the use of contrast media was considered to be contraindicated The diagnosis of chronic pyelonephritis was verified in 50% at biopsy and/or autopsy Hypertension was diagnosed in 8 cases later in the course than the shrivelling of the kidneys at the mean age of 58 1 (42–71) years

Fig 6*B* shows that the mean age of 11 women was 42 9 (30–55) years when in spite of impaired renal function bilateral shrivelling and papillary necrosis were diagnosed by i v urography in the second half of the 1950s and later on an average 4 9 (2–8) years after the initial examination showing no abnormalities The diagnosis of chronic pyelonephritis was verified by renal angiography in 3 and by biopsy and/or autopsy in 5 cases Uraemia developed during the observation period in 4 patients Three fourths had a past history with episodes of acute pyelonephritis The interval between the onset of UTI and the finding of the afore mentioned radiological abnormalities averaged 16 9 (8–34) years in the 9 cases in which the information was certain

sults (2) The impaired concentrating ability in the bacteriuric series increased significantly following eradication of the bacteriuria In some cases the concentrating ability decreased temporarily in association with UTI during the follow up period (2)

A prerequisite for comparable results was that the method of determining the maximal concentrating ability remained constant during the follow up period There were no significant differences in the control series between the mean values obtained initially during the follow up period and finally It should be mentioned that the age related decrease in concentrating ability (3) is not reflected by the said results the change may be too small however to influence the results of the calculations or it may be compensated for by the training in careful preparation that the subjects obtained from repeated tests and by intake of furosemide (3)

In series 4 there were no significant differences between the mean values following the eradication of the bacteriuria and those at the final medical examination All the women were treated and hence there were no controls in the series The result may be accounted for by adequate initial treatment and by the fact that in the continued course of the study UTI was given special consideration and treatment being looked upon as a serious disease Women with pyuria at the initial examination (series 3) on the other hand were probably treated less systematically the final mean value in this series was significantly lower than the initial value The only comment here on the results in series 2 is the statement that the final mean value was significantly higher than the initial value

Occurrence of subjective symptoms of UTI during the follow up and findings of urinary abnormalities (bacteriuria and/or pyuria) at the final examination

The number of women with subjective symptoms during the follow up was significantly lower in the control series 1 than in the other three series as well as in the corresponding subgroups of series 3 and 4 with no past history of UTI these women could thus represent cases at risk The differences between series 2 3 and 4 were not significant It seems interesting that series 2 had a significantly lower number of cases with symptoms than the corresponding subgroup of series 3 with a past history of UTI and initial sterile pyuria but that the difference between series 2 and the corresponding subgroup of series 4 with initial bacteriuria/pyuria was not significant (as a result of the long term treatment of the bacteriuria?)

The number of women with urinary abnormalities (bacteriuria and/or pyuria) at the final examination was significantly lower in series 1 than in the other three series and in the corresponding subgroup of series 3 with no past history of UTI and initial sterile pyuria thus these women could represent cases at risk The difference between series 1 and the corresponding subgroup of series 4 with no past history of UTI and initial bacteriuria/pyuria was not significant (a result of therapy?) The number of cases with urinary abnormalities was significantly higher in series 3 than in series 2 and 4 the number was significantly higher in series 3 than in series 4 The differences between series 2 and the corresponding subgroups of series 3 and 4 were significant These results may be influenced by a possible reduction of the occurrence of urinary abnormalities in bacteriurics (series 4) following treatment of the bacteriuria and by other factors than bacterial infection causing pyuria (series 3) It should be stressed that contrary to the cases in series 4 with initial bacteriuria/pyuria the cases in series 3 with initial pyuria were not treated systematically

At the final medical examination bacteriuria was recorded in a significantly lower number of women in series 1 than in the other series between which there were no significant differences

Accordingly series 2 3 and 4 could represent groups at risk from the viewpoint of UTI Our results of long term treatment of the bacteriuric series do not seem to agree with the following experience and conclusions (6) the development of symptomatic infection was not prevented by a short course treatment (1–2 weeks) such treatment encouraged the development of symptoms especially symptoms of acute pyelonephritis

Occurrence of new cases of hypertension during the follow up

During the follow up a significantly greater number of new cases with hypertension occurred in series 2 3 and 4 than in series 1 There were no significant differences between series 2 3 and 4 The question of the correlation between hypertension and UTI is controversial no significant difference (6 7) and significantly higher BP in bacteriuric women than in non bacteriurics (8)

Final Comments on Population Studies

With a view to the planning of population studies in the future it may be suggested as a working hypothesis that controls selected on the criteria applied in this study will form a relatively homogeneous group with a low risk of UTI and its complications whereas the other series represent a relatively homogeneous group at risk with temporary occurrence of subjective symptoms and urinary abnormalities The overlapping between the groups would then represent cases with UTI without subjective symptoms Such comparisons do not seem possible between bacteriurics and their controls matched for age (7) or age civil state and maternity (6) This question has been discussed elsewhere (3)

Resources for follow up over decades, including treatment not only of bacteriurics but also of pyurics seem to be a prerequisite for further answers to controversial and open questions relating to non obstructive UTI in adult women—and in men (3) for the purpose among others of establishing potential groups at risk of e g com ng hypertension and progressive renal dam

i the long term course and progressive renal damage in selected patients with the diagnosis of chronic UTI

The population study was supplemented by the present author s as yet unpublished clinical studies from an earlier 30-year period The material illustrates the controversial question relating to the presence of progressive kidney damage in non obstructive UTI in normotensive adult women It was found that 29 women mean age 49 0 (26–71) years with no abnormalities on excretion urography 6 1 (1–15) years earlier had bilateral papillary necrosis and/or contracted kidney and impaired kidney function Several of them died in uraemia the diagnosis being pyelonephritis (interstitial nephritis) The mean duration of UTI in the past was 14 7 (2–40) years more than 6 years in 87% and more than 10 years in 47% In 2 women in whom unilateral kidney damage developed radiography at 5–6 years previously had shown no abnormalities and they had a history of UTI for about 15 years Just over two-thirds of the patients had had episodes of acute pyelonephritis The diagnosis of pyelonephritis (interstitial nephritis) was verified by biopsy and/or autopsy in about half the total number of cases

Since progressive renal damage generally developed only after a long history of UTI and at relatively high ages the possibility of such a diagnosis would be limited in population studies with a relatively even age distribution and a follow up period as short as 6–12 years Patients who are undergoing medical treatment are probably disinclined to take part in population studies, and it is therefore important to bear in mind that the material should represent a true cross section of the population Our study does not meet the requirements of being a cross section (3) and of a sufficiently long follow up period Nor do other published studies seem to fulfil these requirements

Hodson had seen neither the development nor progression of pyelonephritis scarring in adult kidneys over periods of 10 to 15 years of follow up (7)

This series of patients was selected from a large clinical material and does not allow any conclusions as to the extent to which chronic UTI is complicated by progressive renal damage demonstrable by i v urography Yet the results show that such a complication can also occur in normotensive women contrary to what has been claimed by others (6 7) Women of upper middle age with long lasting UTI and episodes of acute pyelonephritis seem to be a special group at risk

On the reliability of radiographic methods for excluding the diagnosis of pyelonephritis (interstitial nephritis)

An earlier report includes a case in whom urography showed no abnormalities but renal angiography revealed parenchymal damage with scars (3) Two similar cases are included in the present material

A specially interesting case is that of a woman who had numerous probably acute exacerbations of chronic pyelonephritis and no abnormalities at excretion urography or renal angiography but in whom biopsy showed evidence of chronic pyelonephritis (interstitial nephritis) with an acute inflammatory process Similar diagnostic problems in 7 cases of chronic interstitial nephritis have been reported (9)

To what extent such cases run the risk of progressive kidney damage of clinical importance is still an open question

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Renal Cadmium Concentration in Relation to Smoking Habits and Blood Pressure

Karen Østergaard

From the Department of Pathology Rigshospitalet Institute of Pathology Sundby Hospital Copenhagen and Institute of Pathology Holbæk Hospital Holbæk Denmark

ABSTRACT The cadmium concentration of renal tissue from 120 patients who had died at the age of 45–65 years, has been determined by atomic absorption spectrophotometry The concentration did not differ significantly either between urban and rural dwellers or between male and female subjects The concentration of cadmium in kidneys from cigarette smokers was about twice as high as in kidneys from non smokers Renal cadmium concentration was higher in normotensives than in hypertensives When smoking habits were taken into account, renal cadmium concentration was found to be 82% higher in normotensives than in hypertensives The possible roles of water hardness and selenium intake are discussed

Cadmium has been classified as a non essential trace element It occurs in the environment partly because of certain industrial uses and is present in food tobacco air and water as a contaminant The absorption of ingested cadmium is rather small whereas that of inhaled cadmium is greater The absorbed cadmium is excreted very slowly and it accumulates primarily in the kidneys and the liver (4)

It has been shown (3) that the kidneys contain about 30% and the liver about 20% of the total body burden The biological half life of cadmium in the kidneys has been estimated to about 30 years (8) Renal cadmium concentration (RCC) as determined at necropsy varies with age being nil in the newborn and reaching a maximum at about 50 years (16)

The acute toxicity and the effects of occupational exposure to cadmium have been well documented The possible consequences for public health however of life long exposure to low levels of environmental cadmium are relatively little understood as is the importance of the different sources of cadmium to the general population

The main objectives of this study have been to study the possible relations between cadmium and chronic diseases especially hypertension and the role of smoking habits sex and domicile on the RCC The results may further provide background values for the monitoring of future changes in the cadmium exposure of the general population

MATERIAL AND METHODS

The sampling was carried out consecutively in the period Jan 1974 – Jan 1975 at two Danish hospitals one covering urban districts (Copenhagen) and the other predominantly rural districts (the island of Sealand) To reduce the influence of age on the results only tissue from subjects in the age group 45–65 years was sampled A total of 240 tissue samples from 120 subjects were sampled and analysed The distribution according to age sex and domicile is shown in Table I Information regarding chronic diseases and smoking habits has been obtained from the hospital records

Wedge shaped specimens of renal tissue were excised at autopsies using stainless knives Two tissue specimens each weighing 1–2 g and comprising cortex as well as medulla extending up to the apex of the calyx were sampled from one kidney of each subject

The tissue specimens were kept at −18°C in polystyrene beakers provided with lids until analysis The samples were dried at 100°C in silica crucibles for about 48 hours after which they were ashed at 450°C for approximately 48 hours The ashes were weighed and dissolved in 25 ml of 1 N HNO_3

The solution was analysed for cadmium using an atomic absorption spectrophotometer (Perkin-Elmer model 300 provided with deuterium background corrector) without preceding extraction A $CdCl_2$ solution served as standard The reproducibility of the method is expressed by the deviation from each of the two values as % of the mean $((|c_1-c_2|)/(c_1+c_2)\times 100)$ The mean value for all the duplicate analyses was 7 0%

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Bone Mineral Content in Chronic Renal Failure during Long-Term Treatment with 1α-Hydroxycholecalciferol

S Madsen K Ølgaard and J Ladefoged

From Medical Department P Division of Nephrology Rigshospitalet Copenhagen Denmark

ABSTRACT Bone mineral content of the radius (BMC) has been measured by photonabsorptiometry in 22 adults with chronic renal failure before and after on an average 15 4 months of treatment with 1α hydroxycholecalciferol (1α HCC) Identical measurements were performed in a control group comprising 26 uremic adults, who did not receive any vitamin D supplements during the study The BMC was reduced and not significantly different in the two groups of uremic patients at the initial measurement During treatment with 1α HCC the BMC increased significantly in 3 patients while the total group showed only a small (mean 0 7%) non significant increase In the control group the BMC decreased significantly in 4 patients, while the total group showed a non significant decrease (mean 3 5%) However a comparison of the BMC changes in the two groups revealed a significant difference ($p<0\ 01$) The BMC changes in the 1α HCC group were uncorrelated to changes in serum calcium ionized calcium, phosphate alkaline phosphatases immunoreactive parathyroid hormone and intestinal calcium abosrption during therapy The data indicate that cessation of skeletal demineralization in chronic renal failure may be achieved by 1α HCC but remineralization of the skeleton seems to be attainable only in a minority of adult patients

The development of bone disease in patients with chronic renal failure has received much attention in recent years partly due to the introduction of dialysis which extends the life expectancy of these patients and partly due to improved comprehension of the pathogenesis of renal bone disease In 1970 it was discovered (12) that the kidney is an endocrine organ of essential importance in the synthesis of the metabolically active form of vitamin D 1 25-dihydroxycholecalciferol (1 25 DHCC) Subsequently it was shown that this metabolite is not detectable in the serum of anephric animals (13) or of patients with chronic renal failure (20) Since then several groups have reported on the beneficial effects of 1 25 DHCC (3 7 15) as well as the convenient analog 1α hydroxycholecalciferol (α HCC) (2 6 16) in the treatment of renal osteodystrophy These studies mostly of short duration in a few patients have in the majority of cases demonstrated improvement or normalization of bone related blood biochemistry intestinal calcium absorption radiology and bone histology beside relief of bone pain during treatment However it has not yet been demonstrated whether this new therapy is capable of arresting the accelerated skeletal demineralization in patients with chronic renal failure (5 14)

In the present study photonabsorptiometry was applied to measurement of changes in the appendicular bone mass during long term treatment of 22 patients with 1α HCC The results were compared with changes in a control group of 26 patients with chronic renal failure

PATIENTS AND METHODS

1α-HCC patients

Twenty two patients (12 females and 10 males) aged 24–60 (mean 44 3) were treated with an oral dose (2 0–0 25 μg daily) of 1α HCC (Leo Pharmaceutical Products) for an average of 15 4 months (range 9–29) Six patients received regular hemodialysis 7 initiated hemodialysis during the study and 9 non-dialysis patients had stable renal function during the study (mean GFR 9 9 ml/min range 2–20)

Control group

Twenty six control patients (11 females and 15 males) aged 26–65 (mean 47 0) were studied for an average of 13 5 months (range 8–22) without any vitamin D treatment

Table I *Changes in bone mineral content (BMC) in 22 uremic patients receiving 1α-HCC therapy and in 26 uremic patients not receiving any vitamin D supplements*

A significant difference ($p<0$ 01) in changes between the two groups is demonstrated

No of pats	Sex	Mean age (y)	Duration of study (mo)	BMC (mean ± S D) (g/cm) Initial	Final	BMC change (%)
1α HCC group						
12	♀	45 7	14 8	0 938±0 180	0 942±0 207	+0 43
10	♂	42 7	16 2	1 273±0 227	1 286+0 234	+1 02
22	♀+♂	44 3	15 4	1 090±0 201	1 098±0 219	+0 70
Control group						
11	♀	45 5	12 3	0 909±0 196	0 864±0 170	−4 95
15	♂	48 1	13 8	1 349±0 149	1 312±0 159	−2 74
26	♀+♂	47 0	13 2	1 162±0 169	1 123±0 163	−3 47

The 3 patients with a significant increase in BMC ($p<0$ 05) were treated for 20 19 and 12 months re spectively with 1α HCC The increases amounted to 14 5 13 5 and 11 5% or 8 7 8 5 and 11 5% per year The first two (one female aged 47 with creatinine clearance of 8 ml/min due to chronic in terstitial nephropathy and one male aged 45 receiv ing regular hemodialysis) complained of bone pain at the start of the study and in both patients this symptom vanished during 1α HCC treatment

In the control group the BMC declined in 22 pa tients (range 0 1–13 9%) was unchanged in one and rose in 3 (range 2 9–5 5%) In no case did BMC increase significantly The average decline of BMC during the study was 3 47% (females 4 95 males 2 74%) or 3 09% per year In 4 patients the BMC declined significantly but in the control patients as a group the decline was not significant (Table I) Further the BMC changes were not significantly different in dialysis and non dialysis patients How ever when 1α HCC treated patients and control patients were compared the changes in BMC and BMC/W during the study differed significantly ($p<0$ 01) The average BMC changes are shown in Table I and the intraindividual BMC changes per year in Fig 2

The biochemical and intestinal responses to 1α HCC treatment are summarized in Table II and Fig 3 Serum calcium turned normal in all patients within a month However measurement of serum ionized calcium revealed that the patients in fact were slightly hypercalcemic during the treatment (Fig 3) Serum inorganic phosphate was unchanged during the study Serum alkaline phosphatases which initially were elevated in 11 of the 1α HCC patients declined significantly ($p<0$ 001) and were normal in 20 of the patients at the end of study i PTH was initially elevated in 19 of the patients A significant suppression ($p<0$ 001) of i PTH took place during therapy and 11 patients had normal i PTH at the end of the study The intestinal calcium absorption was initially low especially when oral calcium intake was considered The calcium absorption increased in all 22 1α HCC patients and was at the end of study not signifi cantly different ($p>0$ 1) from the intestinal calcium absorption in 10 normal adults

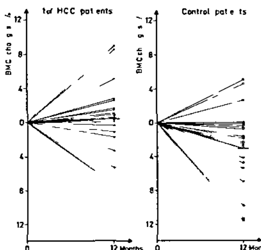


Fig 2 Changes in bone mineral content (%/year) in 22 uremic patients receiving 1α HCC therapy (to the left) and in 26 uremic patients not receiving any vitamin D supplements (to the right) Mean change is indicated by the thicker line

Table II *Bone related parameters (mean ± S E M) in 22 uremic patients during treatment with 1α-HCC*

Duration of treatment (mo)	Serum calcium (mmol/l)	Serum ionized calcium (mmol/l)	Serum phosphate (mmol/l)	Serum alkaline phosphatases (U/l)	i PTH (ng/ml)	Intestinal Ca absorption (%)
-	2 20±0 05	0 95±0 04	1 74±0 08	355±79	7 1±1 0	10 9±1 0
3	2 49±0 03	1 10±0 03	1 74±0 08	298±66	4 6±0 5	21 2±2 5
6	2 57±0 02	1 16±0 02	1 82±0 08	214±30	3 6±0 4	-
15 4	2 59±0 02	1 16±0 02	1 77±0 09	195±23	2 8±0 3	18 0±1 2

DISCUSSION

Photonabsorptiometry provides an accurate non invasive and easy quantification of bone mass making the technique very suitable in the evaluation of therapeutic attempts to restore the mineralization of the skeleton in various metabolic bone disorders Whole body neutron activation technique (9) may be the most accurate technique available for measuring changes in total body calcium in vivo (8) However very expensive and elaborate equipment is required and it has furthermore been demonstrated (10) that results from BMC measurements the radius in normal adults as well as in patients with chronic renal failure are correlated to the results from neutron activation analyses

In a large patient series we recently demonstrated (18) by photonabsorptiometry on cortical radius that patients with chronic renal failure during an accelerated bone loss (approximately 3% per year) develop a significantly reduced bone mass In accordance with this finding both groups of patients in the present investigation had low BMC values initially In the 1α HCC patients as a group the BMC did not increase significantly during therapy but it was striking that the BMC changes were significantly different from the continued bone loss seen in non treated uremic patients This finding indicates that cessation or reduction of skeletal demineralization in patients with chronic renal failure may be achievable with 1α HCC treatment However remineralization of the skeleton in uremic patients seems in accordance with our previous study (19) to be attainable only in a minority of patients

A recent study (21) suggests that increases in whole body calcium during 1α HCC treatment are achieved only in patients in whom biochemical and histological improvements occur during treatment The present study cannot confirm this Bone biopsies were not performed in the present investigation but careful examination of the biochemical data revealed no difference in biochemical response to 1α HCC whether BMC increased or decreased during the treatment

The present data indicate that 1α HCC apart from being effective in normalizing bone related blood biochemistry and promoting intestinal calcium absorption may also terminate the accelerated bone loss in patients with chronic renal failure

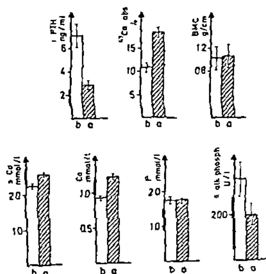


Fig 3 Bone related parameters before (*b*) and after (*a*) long term treatment (15 4 months) with 1α HCC Normal values are indicated on the ordinates Ī = Mean ± S E M Ca^{++}=serum ionized calcium P_i=serum inorganic phosphorus *s alk phosph* =serum alkaline phosphatases *i PTH*=serum immunoreactive parathyroid hormone *⁴⁷Ca-abs* =intestinal absorption of calcium *BMC*=bone mineral content of cortical radius

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Table II *Bone related parameters (mean ± S E M) in 22 uremic patients during treatment with 1α-HCC*

Duration of treatment (mo)	Serum calcium (mmol/l)	Serum ionized calcium (mmol/l)	Serum phosphate (mmol/l)	Serum alkaline phosphatases (U/l)	i PTH (ng/ml)	Intestinal Ca absorption (%)
–	2 20±0 05	0 95±0 04	1 74±0 08	355±79	7 1±1 0	10 9±1 0
3	2 49±0 03	1 10±0 03	1 74±0 08	298±66	4 6±0 5	21 2±2 5
6	2 57±0 02	1 16±0 02	1 82±0 08	214±30	3 6±0 4	–
15 4	2 59±0 02	1 16±0 02	1 77±0 09	195±23	2 8±0 3	18 0±1 2

DISCUSSION

Photonabsorptiometry provides an accurate non invasive and easy quantification of bone mass, making the technique very suitable in the evaluation of therapeutic attempts to restore the mineralization of the skeleton in various metabolic bone disorders Whole body neutron activation technique (9) may be the most accurate technique available for measuring changes in total body calcium in vivo (8) However very expensive and elaborate equipment is required and it has furthermore been demonstrated (10) that results from BMC measurements the radius in normal adults as well as in patients with chronic renal failure are correlated to the results from neutron activation analyses

In a large patient series we recently demonstrated (18) by photonabsorptiometry on cortical radius that patients with chronic renal failure during an accelerated bone loss (approximately 3% per year) develop a significantly reduced bone mass In accordance with this finding both groups of patients in the present investigation had low BMC values initially In the 1α HCC patients as a group the BMC did not increase significantly during therapy but it was striking that the BMC changes were significantly different from the continued bone loss seen in non treated uremic patients This finding indicates that cessation or reduction of skeletal demineralization in patients with chronic renal failure may be achievable with 1α HCC treatment However remineralization of the skeleton in uremic patients seems in accordance with our previous study (19) to be attainable only in a minority of patients

A recent study (21) suggests that increases in whole body calcium during 1α HCC treatment are achieved only in patients in whom biochemical and histological improvements occur during treatment The present study cannot confirm this Bone biopsies were not performed in the present investigation but careful examination of the biochemical data revealed no difference in biochemical response to 1α HCC whether BMC increased or decreased during the treatment

The present data indicate that 1α HCC apart from being effective in normalizing bone related blood biochemistry and promoting intestinal calcium absorption may also terminate the accelerated bone loss in patients with chronic renal failure

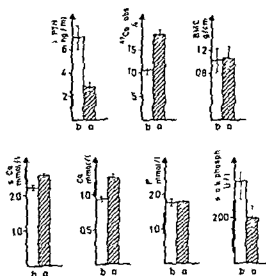


Fig 3 Bone related parameters before (*b*) and after (*a*) long term treatment (15 4 months) with 1α HCC Normal values are indicated on the ordinates Ī = Mean ± S E M *Ca⁺⁺*=serum ionized calcium *Pi*=serum inorganic phosphorus *s alk phosph* =serum alkaline phosphatases *i PTH*=serum immunoreactive parathyroid hormone *47Ca-abs* =intestinal absorption of calcium *BMC*=bone mineral content of cortical radius

ACKNOWLEDGEMENT

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Acta Med Scand 203 391–398 1978

Organization and Efficacy of an Out-Patient Hypertension Clinic

Ove Andersson Goran Berglund Lennart Hansson Rune Sannerstedt
Ramon Sivertsson John Wikstrand and Lars Wilhelmsen

From the Department of Medicine I Sahlgrenska Hospital University of Goteborg Goteborg Sweden

ABSTRACT A description is given of the diagnostic and therapeutic routines at an out patient hypertension clinic The efficiency of treatment in terms of BP control patient adherence and frequency of side effects is presented The patients were derived from a screening examination of a random third of an urban male population aged 47–54 years Cut off points for hypertension were BP above 175 (systolic) or 115 (diastolic) mmHg on two separate occasions or current antihypertensive therapy With these criteria 11 2% of the population was considered hypertensive Out of 686 hypertensive men cared for at the clinic only 4 2% defaulted during a two-year follow up At the second annual examination 83% had casual diastolic BP below 105 mmHg with an average reduction of 10 mmHg The antihypertensive therapy consisted of single drugs, a diuretic or a β-adrenergic blocking agent in 32% of the patients Fifty per cent of the patients had a combination of two drugs diuretics and β adrenergic blockers or β-adrenergic blockers and hydralazine while 14% had the triple combination of diuretics, β adrenergic blockers and hydralazine Side-effects so serious as to cause withdrawal of a drug occurred in 15% of the patients during the first year and in 3% during the second year of antihypertensive therapy

Arterial hypertension is recognized as an important risk factor for cardiovascular disease (12 13 22) Antihypertensive therapy prevents some of these complications as shown in a controlled study (17) Epidemiological studies indicate that 10–15% of middle aged men have hypertension in need of treatment and that most of them are either untreated or inadequately treated (5 10 11 16) This is the background to the growing interest in procedures aimed at finding and treating a larger proportion of the hypertensive subjects in the population However the fact that many patients with known hypertensive disease drop out of treatment (1 2 6 7 10 14 19) is disappointing The creation of rational out patient units might be a tool for achieving more effective treatment and supervision of an often life long therapy but so far only scattered results from such units have been presented (4 9 14)

This paper describes an out patient hypertension clinic and presents long term results of BP reduction and frequency of side effects and drop-outs in a random population sample of hypertensive middle aged men

THE HYPERTENSION CLINIC

Objectives and organization

An out patient clinic for hypertensive patients was started at Sahlgren s Hospital in 1970 The main objectives were To treat the hypertensive patients in an ongoing primary preventive trial (21) To be a referral centre for patients with suspected secondary hypertension and for patients with hypertension responding inadequately or with serious side-effects during hypertensive treatment To constitute a research unit for epidemiological clinical and experimental studies on arterial hypertensive disease

The Hypertension Clinic is located in the out patient premises of the Department of Medicine I In 1971 the full time staff consisted of three specially trained nurses and one secretary All BP measurements collecting of blood samples as well as part of the history taking were done by the nurses The secretary had a key role in administering the follow up She also contacted patients who had failed to attend to follow-up in order to arrange new appointments Nine doctors with a special interest in arterial hypertensive disease ran one half-day clinic each The patients saw the same doctor or nurse on each visit The clinic worked almost exclusively on an out patient basis When indicated however patients could be hospitalized

PATIENTS AND SCREENING METHODS

The number of patients and visits in 1972–76 are shown in Table I On Dec 31 1975 1 822 patients were under care

Table 1 *Number of patients and visits to physicians and nurses at the Hypertension Clinic during 1972–76*

Year	No of pats	No of visits		
		To physicians	To nurses	Total
1972	650	2 215	2 368	4 583
1973	1 150	2 859	3 278	6 137
1974	1 550	3 246	5 009	8 255
1975	1 822	3 402	4 207	7 609
1976	1 585	3 101	3 566	6 667

at the clinic About 50% of them were recruited from the random population sample while the rest were referred from physicians The decrease in the number of patients and visits during 1975–76 was due to an increased re referral of patients to the referring physician after diagnostic examination and/or treatment The hypertensives from the random population sample were kept at the clinic for further follow up The following presentation is confined to the hypertensives recruited from the initial BP screening examination in the random population sample This screening was part of a multifactor primary prevention trial started in 1970 (21) The intervention group consisted of a randomly selected third of all men aged 47–54 years at entry (born 1915–22 and 1924–25) and living in Goteborg =9996) Men born in 1923 were not included as the ts in this age group took part in another study Of 996 subjects invited 7455 (75%) attended the screen examination which included determination of BP oking habits and serum cholesterol The screening examination was performed in the hospital after a working day between 4 30 and 7 00 p m The patients were referred to the Hypertension Clinic when the systolic blood pressure (SBP) was above 175 mmHg or the diastolic blood pressure (DBP) was above 115 mmHg at the screening and at a re-examination after two weeks or if the patient already was on antihypertensive treatment Patients with SBP above 230 or DBP above 140 mmHg at screening were immediately referred to a department of internal medicine for diagnostic work up and treatment Subjects with SBP of 160–174 mmHg or DBP of 95–114 mmHg at the first or second examination were invited to a BP examination after one year

Of the screened population 835/7455 subjects (11 2%) were regarded as hypertensives according to the criteria mentioned Of these 835 patients 686 (82%) took part in the diagnostic examination and follow up at the hypertension clinic The remaining 149 patients preferred treatment by other physicians or refused to participate Of the 686 hypertensives 270 (39%) were on treatment at the screening examination while 416 (61%) were untreated The prevalence of secondary hypertension (5 7%) has been reported elsewhere (5)

Blood pressure measurements

The routine BP measurements at the Hypertension Clinic on the right arm and recorded to the nearest 2 mmHg were performed by the nurses A rubber cuff 12 cm wide and 26 cm long connected to a mercury manometer was used The BP was registered in the supine position after 5 min rest and after 1 min in the standing position DBP was recorded as phase 5 i e when the sounds disappeared The heart rate was measured immediately before the BP measurements in the supine position The BP at the screening examination was determined by a physician after a 4–5 min long interview concerning the subject s physical health the measurement was made with the same technique as described above but with the subject sitting

Diagnostic examination

All patients underwent the same examinations These were done stepwise and started with two visits to the nurses for measurement of BP and heart rate usually with a fortnight between the readings During this time chest X ray ECG and samples for blood tests (Hb ESR serum electrolytes creatinine bilirubin alkaline phosphatases GOT and GPT uric acid cholesterol and triglycerides) and urine tests (protein glucose sediment and osmolality after 13 hours thirst) were taken At the third visit to the clinic the patients were examined by a physician who decided whether further diagnostic examinations were needed Drugs were prescribed and plans were outlined for further management Data from this third visit were used for comparison with follow up data

After the initial phase patients with uncomplicated and adequately controlled hypertension saw the doctor once or twice a year BPs were controlled by the nurses at 3–6 monthly intervals For patients with problems of any kind the intervals between controls were adapted individually If needed the patient could get in touch with his physician via the secretary at the clinic

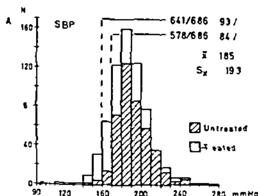
Data were recorded in a standardized manner for scientific purposes Special forms were used for direct coding and computerization

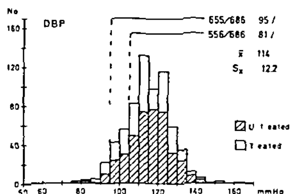
Indications for antihypertensive therapy

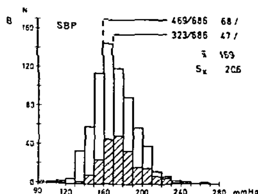
Antihypertensive treatment was introduced in 1) Patients between 40 and 60 years with repeated casual BP above 170/105 at rest Under the age of 40 and above the age of 60 the corresponding BP levels have been 160/95 and 180/110 respectively The same BP limits were used for men and women 2) Patients with BPs below the above mentioned limits if hereditary factors hypertensive organ manifestations or other risk factors for cardiovascular disease implied an increased risk These decisions have been made at the discretion of the physician

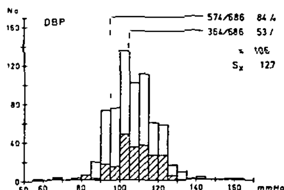
The aim of the antihypertensive therapy was to reduce the BP to what has been considered normal for the patient i e under the age of 40 a BP below 150/90 mmHg between 40 and 60 years of age below 160/95 mmHG and above 60 years of age below 170/105 mmHg

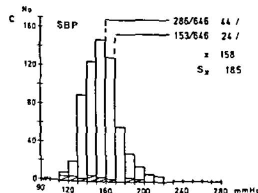
Fig 1 Systolic (*SBP*) and diastolic BP (*DBP*) distribution for treated and untreated patients at screening (*A*) initially at the Hypertension Clinic (*B*) and after one (*C*) and two years treatment (*D*) Means and S D are also given as well as the frequency of patients exceeding 160 and 170 mmHg (SBP) and 95 and 105 mmHg (DBP) respectively

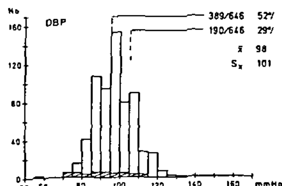

A
SBP
641/686 93 %
578/686 84 %
x̄ 185
S_x 19.3
Untreated
Treated
mmHg

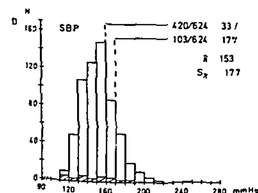

DBP
655/686 95 %
556/686 81 %
x̄ 114
S_x 12.2
mmHg


B
SBP
469/686 68 %
323/686 47 %
x̄ 169
S_x 20.6
mmHg


DBP
574/686 84 %
364/686 53 %
x̄ 105
S_x 12.7
mmHg


C
SBP
286/646 44 %
153/646 24 %
x̄ 158
S_x 18.5
mmHg


DBP
389/646 52%
190/646 29%
x̄ 98
S_x 10.1
mmHg


D
SBP
420/624 33 %
103/624 17%
x̄ 153
S_x 17.7
mmHg

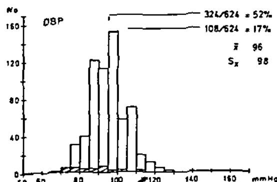

DBP
324/624 = 52%
108/624 = 17%
x̄ 96
S_x 9.8
mmHg

Table II *Mean (x) and S D of systolic (SBP) and diastolic BP (DBP) at screening initially at the Hypertension Clinic and after one and two years treatment*

Only patients followed during the whole observation period and on antihypertensive therapy are included (n=589)

	SBP		DBP	
	x	S D	*x*	S D
At screening	184	19 1	114	12 0
Initially at the clinic	168	20 1	106	12 4
After one year	158	18 2	97	9 8
After two years	153	17 7	96	9 8

Choice of antihypertensive drugs

The first drug of choice has been β adrenoreceptor blocking agents in doses corresponding to propranolol 80–640 mg/day Thiazide diuretics and chlorthalidone have also been common preferably in elderly patients with severe hypertension in whom cardiac decompensation was found or anticipated Diuretics have been used in doses corresponding to 25–75 mg of hydrochlorothiazide per day We have generally preferred a combination of drugs rather an undue increase in the dose of a single drug If nor ion was not achieved with the initially given β blocker and/or a diuretic hydralazine (75– mg/day) has been added If the combination of these did not result in normotension spironolactone bethanidine or occasionally high-dose furosemide or minoxidil have been added

Registration of adverse drug effects

Withdrawals of drugs on account of supposed side-effects were registered at the end of the first and second year A retrospective analysis of a possible causal relationship between drugs and adverse effects was made by checking the case records The judgement of causality was based on laboratory tests subjective symptoms or signs related in time to the institution of treatment and recovery after withdrawal Provocation tests were not used The analysis was done by one of the authors (O A)

Statistical methods

Standard methods were used for the calculation of means and S D The hypothesis of no difference between means was tested with Student s *t* test The hypothesis of no difference between proportions was tested with the χ^2 test Only two-sided tests were used Differences were regarded significant for $p<0\ 05$

RESULTS

Blood pressure

Fig 1 presents the distribution of SBP and DBP in treated and untreated patients at the screening examination at the second visit to the Hypertension Clinic and after one (n=646) and two years (n=624) treatment The discrepancy in number was caused by patients who were lost to follow up The distributions of SBP and DBP initially and after one and two years treatment were gradually shifted to the left The proportion of patients with SBP or DBP above 160/95 mmHg decreased significantly from 68% (SBP) and 84% (DBP) initially at the Hypertension Clinic to 33 and 52% after two years treatment Correspondingly the proportion of subjects with SBP or DBP above 170/105 mmHg decreased from 47% (SBP) and 53% (DBP) to 17% (SBP) and 17% (DBP) The average BP also decreased from 168/106 mmHg initially to 153/96 mmHg after two years treatment

When only patients on drug treatment were included (n=589) there was still a significant reduction of SBP (15 mmHg) and DBP (10 mmHg) during two years follow up (Table II) The major part of the reduction was achieved during the first year

The proportion of patients on antihypertensive treatment increased from 39% at the screening examination to 68% initially at the Hypertension Clinic and to 94% after one and two years treatment The increase in the proportion of treated patients from the screening examination to the third visit to the Hypertension Clinic was due to initiation of treatment during the phase of diagnostic investigations During the screening of men born in

Table III *Type of treatment after one and two years follow up*

	After one year (n=610)		After two years (n=589)	
	No	%	No	%
Single drugs				
Adrenergic β-blockers	122	20	106	18
Diuretics	110	18	82	14
Combinations				
Adrenergic β-blockers + diuretics	144	24	153	26
Adrenergic β blockers + hydralazine	149	24	141	24
Adrenergic β-blockers + diuretics + hydralazine	58	10	84	14
Other drugs or drug combinations	27	4	23	4

1915–17 those considered hypertensive were put on treatment prior to the work up at the clinic In the younger age cohorts such treatment was instituted only in patients with very high BPs considered in need of immediate treatment

Type of treatment

After one year s treatment at the clinic, 38% of the treated patients ($n=610$) were on a single drug regimen and 62% on a combination of drugs (Table III) The single drugs most commonly used were β-adrenoreceptor blocking agents (20%) and diuretics (18%) while the dominating drug combinations were β adrenoreceptor blocking agents + diuretics (24%) and β adrenoreceptor blocking agents + hydralazine (24%) Ten per cent were on a triple drug combination with β adrenoreceptor blocking agents + diuretics + hydralazine while 4% were on other drugs or drug combinations Thus altogether 473/610 (78%) were on β adrenoreceptor blocking agents and 312/610 (51%) on diuretic agents 74% of whom had potassium supplement

Table IV *Frequency of adverse effects leading to withdrawal of the drug during the first and second year of treatment*

	First year		Second year	
	No	%	No	%
Diuretics	37/312	12	9/319	3
Diabetes mellitus	4		1	
Gastrointestinal symptoms	4	4	1	1
Gouty arthritis	1		2	
Others	3		–	
S-potassium <3 4 mmol/l	18	8	4	2
S-uric acid >500 mmol/l	7		1	
β-adrenergic blocking drugs	17/473	4	6/484	1
Sleep disturbances	5		3	
Pulmonal obstructive symptoms	4		1	
Gastrointestinal symptoms	2		1	
Bradycardia	1		1	
Intermittent claudication	1		–	
Others	4		–	
Hydralazine	2/207	1	0/225	
Arthritis	2			

Table V *Reasons for drop out during two years at the Hypertension Clinic (n=686)*

	First year		Second year	
	No	%	No	%
Refused or lost for unknown reasons	18	2 6	11	1 6
Preferred treatment by other physicians	9		2	
Moved	7		4	
Died	6		5	
Total	40	5 8	22	3 2

Between the first and second annual examination there were only minor changes in the type of treatment (Table III)

Adverse effects

In 90/610 patients (15%) during the first year and in 15/589 (3%) during the second an antihypertensive agent had to be discontinued because of supposed adverse effects In another 12 patients (8 during the first and 4 during the second year) a relationship between the drugs and the adverse effects was considered improbable These 12 patients were not included in the analysis below

The reasons for withdrawing the three antihypertensive agents most commonly used during the first and second years are listed in Table IV During the first year 37/312 patients (12%) had to discontinue their diuretic treatment A low level of serum potassium in spite of potassium supplement was the most common reason Serum uric acid levels exceeded 500 mmol/l in seven patients and one patient developed clinically overt gout Four patients had to discontinue the treatment because of gastrointestinal symptoms and four developed clinically overt diabetes mellitus with glucosuria One patient developed gouty arthritis considered secondary to diuretic treatment Three patients complained of unspecific symptoms and wanted to change the diuretic drug

During the first year 17/473 patients (4%) had to discontinue β adrenergic blocking treatment In five patients this was due to vivid dreams and insomnia Pulmonary obstructive symptoms was the reason in four patients and gastrointestinal symptoms in another two One patient developed bradycardia and another experienced worsened symptoms from intermittent claudication Four pa

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emphasizes the need for frequent appointments with a nurse or a physician and the possibility of telephone contact in the early phase of anti hypertensive treatment On the other hand fewer side effects were registered during the second year of treatment indicating that less close supervision is necessary after the initial treatment phase The β adrenoreceptor blocking drugs caused fewer adverse effects than diuretics This might however be due to the fact that diuretics were more often prescribed to patients with advanced hypertensive disease in whom a higher frequency of side effects per se might be anticipated Further more the frequency of adverse effects with diuretics would also have been considerably reduced if a somewhat lower level of S potassium had been accepted

The number of patients treated at the Hypertension Clinic and the number of visits to nurses and physicians are at present considered maximal (Table I) In fact the reduction of the total number of visits to the clinic during 1975–76 reflected our efforts to increase the intervals between check ups Our experience suggests that a team of one full time nurse one part time secretary and one or more part time physicians can cope with the management of about 500 hypertensive patients of the kind studied here However if a greater proportion of patients with severe hypertension were included as in most out patient hypertension clinics the greater need for diagnostic and therapeutic efforts would probably call for more personnel resources

It must be kept in mind that the problem of treatment of hypertension cannot be solved by out patient hypertension clinics of the kind described here The prevalence of hypertension is so high that the majority of hypertensive patients must be managed by general physicians However the present study has shown that hypertension can be acceptably treated in the majority of detected patients with a low drop out rate and an acceptable frequency of side effects Furthermore the methods used are simple and could probably have wider applicability

ACKNOWLEDGEMENTS

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Validity of Casual Blood Pressure in Women

Thomas Thulin

From the Unit for Community Care Sciences Dalby and the Department of Internal Medicine University of Lund Lund University Hospital Lund Sweden

ABSTRACT In order to assess the prognostic significance of causal BP recordings 64 women not treated for hypertension were selected from a population survey and their BP was followed for six years In addition, a number of parameters considered to be of interest in connection with hypertension were examined The two age-matched study groups comprised women with pressures above the 95th percentile and those with pressures below the 30th percentile respectively The differences in BP between the two groups persisted at repeated casual BP measurements three and six years after the initial examination Similar differences in BP between the two groups were also found after rest The casual BP measurement predicted future BP readings with about the same accuracy as the BP measurement after rest The subjects in the high BP group were significantly more obese and had significantly higher B-hematocrit fS-triglycerides and S-urate levels than those in the low BP group The latter differences were not related to the BP level, but to the degree of obesity The heart rate was significantly higher and codable ST and T wave changes in the ECG were present more often in the group above the 95th percentile This study has shown that women, like men with moderately high BPs have significantly higher levels of some parameters considered to be risk factors in connection with hypertension

It has been established that men with hypertension have an increased risk of developing cardiovascular disease (20 25 26) Moreover they have a higher heart rate are more obese and have higher B hematocrit fS triglycerides and S urate values than normotensive subjects (5 11 24)

Also in women a high BP seems to be associated with an increased cardiovascular morbidity and mortality (4 20) although the risks appear to be less than in men (21) It is well documented that treatment of hypertension improves the prognosis in men (25 26) but rather little is known about the outcome of treatment in females On the whole the problems associated with BP have been studied much more extensively in male than in female subjects

In the present study a group of apparently healthy women with a high BP level in relation to the population in general was compared to another group of age matched females with low BP levels from the same population Initial BP readings were made in 1969–70 and subsequent readings in 1972 and 1975 In addition the ECG and some anthropometric and biochemical parameters were studied

STUDY GROUPS

The subjects were selected from participants in a general ophthalmological survey (3) performed in the Dalby community in 1969–70 In that study and in the present one the BPs were measured in a strictly standardized fashion as described previously (22) The survey comprised 790 females not under treatment for hypertension The 36 women with BPs above the 95th percentile (group A) were matched according to age with 36 women selected from those with BPs below the 30th percentile (group B)

The subjects in group A were selected on the basis of systolic BP (SBP) and diastolic BP (DBP) and pulse pressure The subjects were included in group A if any of these indices or any additive combination thereof exceeded the 95th percentile in the whole population for the five age groups 30–39 40–49 50–59 60–69 and 70–79 years of age Subjects were assigned to group B if the SBPs and DBPs both fell below the 30th percentile The pulse pressure was not used as a selection criterion for group B in order to avoid inclusion of females with low pulse pressures in combination with elevated DBP

The individuals below 30 and above 80 years of age were few and were excluded for this reason In group A one subject refused to participate one failed to attend all investigations one was on antihypertensive treatment and one had developed diabetes mellitus after the first examination in 1969–70 These four subjects and their matched controls in group B were excluded Thus each

Requests for reprints to T Thulin MD The Unit for Community Care Sciences S-240 10 Dalby Sweden

group finally consisted of 32 females None had any (other) apparent disease or was taking any drugs known to affect the parameters studied The mean age (±S D) in 1972 in groups A and B was 49±13 and 49±14 years respectively

METHODS

The follow-up investigations were performed during Jan –May and Sept –Dec in 1972 and during Jan –May in 1975 The subjects fasted and had been requested not to smoke for at least 10 hours before blood sampling

The height and weight were measured according to the WHO recommendations (19) Body surface area was calculated as weight$^{0.425}$ (kg) × height$^{0.725}$ (cm) × 71 84 (9) Relative body weight was calculated as weight (kg)/(height (cm)−100) and the index of obesity (28) as weight (kg)/height^3 (m) This quantity will be referred to as W/H^3 Arm circumference was measured at the middle of the BP cuff and recorded to the nearest 0 5 cm

The B hematocrit fB-glucose fS-cholesterol fS-triglyceride and S-urate levels were analysed by routine methods used at the Department of Clinical Chemistry Lund University Hospital

BP was measured in the morning (8–9 a m) with a mercury manometer on the right arm before and after 10–15 min rest in the recumbent position In 1972 the BP was measured in the same way and in addition after 1 min in the upright position When the arm circumference was more than 30 cm an arm cuff with a rubber bladder measuring 15×43 cm was used Otherwise the standard cuff with a rubber bladder of 12×35 cm was used SBP and DBP (phase 5) were read to the nearest 5 mmHg (1 mmHg≈0 133 kPa) All BPs were measured by the author Mean arterial BP (MAP) was estimated as DBP+(SBP−DBP)/3 The heart rate was calculated as beats/min from the radial pulse during 30 sec The room temperature was 21–23 C

The ECGs were recorded in 1972 with 12 standard leads (I II III aVR aVL aVF V_1 V_2 V_3 V_4 V_5 and V_6) on a three-channel direct writing cardiograph (Elema Schonander) after rest in the recumbent position The ECGs were coded according to the Minnesota Code (19) by two observers (in co-operation with Dr O Werner) When different codes were given the observers discussed the tracing to reach agreement The R wave amplitude in lead V_5 was measured on the last ordinary beat in the record from each subject

Statistical methods

All data were recorded on a previously prepared form and were later transferred to punched cards and verified

Standard statistical methods were used to determine mean values standard deviations and correlation coefficients The majority of the calculations were performed at the Computer Centre in Lund Statistical analyses were also made by using an Olivetti Programma 101 The hypothesis that no differences existed between two groups was checked with Student s t test on the differences within matched pairs Intra-individual differences within a group were also tested by the t test for paired data. Correction for weight was carried out with linear regression

Table I *Casual blood pressures (mmHg) in groups A and B at the first study in 1969–70 and at the follow up examinations in 1972 and 1975 (M±S D)*

	Group A	Group B
SBP		
1969–70	175±34	114±8
1972	190±34**	130±15***
1975	179±32	123±19**
DBP		
1969–70	102±13	73±6
1972	103±14	76±7**
1975	97±13*	73±7

Significant difference compared with values from 1969–70 *$p<0.05$ **$p<0.01$ ***$p<0.001$

Conversion from traditional units to SI units 1 mmHg ≈0 133 kPa

The significance levels were expressed as * 0 01<p<0 05 ** 0 001<p<0 01 ***p<0 001 The following symbols were used N=number M=mean S D =standard deviation n s =not statistically significant (p>0 05) All values are given as M±S D unless otherwise stated (Statistical advice was given by B Sjostedt and B Zoger)

RESULTS

Casual blood pressures at the time of selection and at follow up

The results are given in Table I The differences in SBP and DBP between the two groups persisted from the time of selection in 1969–70 to the follow up examinations in 1972 and 1975

The mean SBP was higher in 1972 than at the time of the initial recordings in both groups (group A p<0 01 group B p<0 001) In 1975 the SBP had decreased (group A p<0 01 group B p<0 01) and group A again had the same level as initially while group B still showed a significantly higher (p<0 01) SBP level

The DBP varied less between the different occasions It was somewhat lower in group A in 1975 than in 1969–70 (p<0 05) and higher in group B in 1972 than in 1969–70 (p<0 01) (Table I)

Blood pressure after rest at follow up

The SBPs in 1972 were 170±29 and 120±14 mmHg and the DBPs 96±13 and 74±7 mmHg in groups A and B respectively After rest SBP decreased significantly between 1972 and 1975 in groups A (p<0 05) and B (p<0 001) while DBP remained

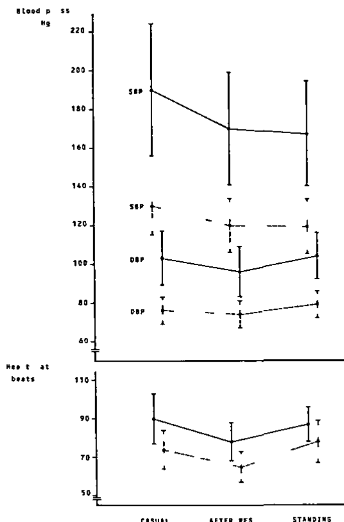


Fig 1 Systolic (*SBP*) and diastolic (*DBP*) BPs and heart rates (M+S D) before and after rest and in the standing position in group A (—) and in group B ( ) Conversion from traditional units to SI units 1 mmHg ≈ 0 133 kPa

unchanged in group A but decreased significantly ($p<0\ 001$) in group B (Table II)

Comparison between casual BP and BP after rest

The results obtained in 1972 are shown in Fig 1 SBP decreased significantly after rest in both groups ($p<0\ 001$) both in 1972 and in 1975 DBP decreased significantly after rest in groups A ($p<0\ 001$) and B ($p<0\ 01$) in 1972 and also in 1975 ($p<0\ 05$ and $p<0\ 01$ respectively)

Reliability of casual BP measurements and those after rest

The intra individual change in BP measurements between 1972 and 1975 was also studied and recorded for each subject It appears from Table II that the decrease between 1972 and 1975 was of the same magnitude for casual and resting BPs If the standard deviations in Table II are taken as measures of the *reliability* of the BP measurements it would seem that almost no differences in reliability exist between casual and resting BP measurements

Table II *Intra individual change in blood pressure (mmHg) between 1972 and 1975 (M+S D)*

	SBP		DBP	
	Casual	Resting	Casual	Resting
Group A	11+?0	8+18	6+11	1+11
Group B	7+13	8+10	4+ 7	4+ 6

Conversion from traditional units to SI units 1 mmHg ≈ 0 133 kPa

Table III *Anthropometric and chemical measurements in 1975 in the two groups (M±S D)*

	Group A	Group B	Significance of the differences between groups
Height (cm)	160±6	162±6	n s
Weight (kg)	78.8±16.4	60.4±9.5	***
BSA (m^2)	1.78±0.18	1.63±0.12	**
Arm circumference (cm)	30.3±4.0	27.1±2.8	***
Relative body weight	1.25±0.28	0.97±0.17	***
Index of obesity	18.3±4.1	14.1±2.5	***
fS-cholesterol (mmol/l)	5.9±1.1	5.5±1.0	n s
fB glucose (mmol/l)	4.8±0.4	4.6±0.5	n s
B hematocrit (%)	39±2	37±3	***
fS-triglycerides (mmol/l)	1.10±0.67	0.77±0.59	*
S urate (μmol/l)	257±51	228±56	*

* $p<0.05$ ** $p<0.01$ *** $p<0.001$ n s =not significant

This was true for both groups and for both the SBP and the DBP

Blood pressure in the standing position

The SBP did not change significantly from rest to standing in either group while the DBP increased in both groups ($p<0.001$) (Fig 1)

Heart rate

— results obtained in 1972 are given in Fig 1 In A the mean heart rates before as well as after and in the standing position were significantly igher ($p<0.001$ or $p<0.01$) than in group B The heart rate decreased after rest in both groups ($p<0.001$) When the casual heart rate and the heart rate after rest were compared in 1972 and 1975 it was found that the rate after rest had decreased by 12 and 9 beats/min respectively in group A and by 9 and 7 beats/min respectively in group B The heart rate increased from rest to the standing position by 9 beats/min in group A and by 13 beats/min in group B

ECG at rest

No subject had codable Q waves Extreme left axis deviation and high R wave amplitudes did not differ statistically between the two groups (three and two cases respectively in each group) There was no difference in the R wave amplitude in lead V_5 between the groups (1.4±0.6 mV in group A and 1.3±0.6 mV in group B) Codable ST changes were found in six cases in group A and in two cases in group B T wave changes were observed in seven cases in group A and in one case in group B One subject in group A had a left bundle branch block and one in group B had atrial fibrillation

Anthropometric measurements

The results obtained in 1975 are given in Table III The mean values of weight arm circumference relative body weight and W/H^2 were all significantly higher ($p<0.001$) in group A than in group B There was no significant difference in height The body surface area was larger ($p<0.01$) in group A than in group B All these measures were virtually unchanged between 1972 and 1975

Chemical parameters

The subjects in group A had significantly higher B hematocrit fS triglyceride and S urate levels than the subjects in group B There was no difference between the two groups in fB glucose or fS cholesterol levels The results obtained in 1975 are shown in Table III Virtually the same differences between the groups were seen both in 1972 and in 1975 although the actual levels were not exactly the same The differences between the groups in the above mentioned parameters disappeared after correction for the calculated influence of obesity

DISCUSSION

It is not known whether drug treatment improves the prognosis in symptomless hypertensive women even if they have moderately elevated BP levels (18) Particularly in the elderly the possible gain of treatment must be weighed against the side-effects (12) In the present study very high BPs (over

200/110 mmHg) were found only in persons aged above 60 years Therefore and as they were all asymptomatic it was considered justified not to treat them

In group A with the higher BPs the mean weight was significantly higher ($p<0\ 001$) than in group B with the lower BPs Moreover the females with the high BP levels in relation to the population in general were more obese as expressed by the W/H^3 and relative body weight than females with low BPs Similar results have previously been reported in males and females with hypertension (7 10 24)

The size of the arm cuff was adjusted to the arm circumference This seems to be especially important in females (10) in order to avoid falsely high BP readings on thick arms

Whyte (27) has proposed that an increased body size demands an increased cardiac output resulting in higher BP and heart rate Therefore it was investigated whether the differences in body size could explain the difference in BP between the two groups It was assumed that the peripheral resistance was equal in the two groups and that the differences in BP could be explained by differences in cardiac output caused by an excess of adipose tissue in group A The cardiac output in group B was set to 5 7 l/min according to Barrat Boyes and Wood (2) Blood perfusion in one kg adipose tissue which comprises about 64% fat 22% cell residue and 14% extracellular water (6) was set to 26 ml/min (14) The subjects in group A were on an average 14 5 kg heavier than those in group B Since the mean heights were equal the excess weight was assumed to consist of adipose tissue This gave an excess cardiac output in group A of 0 4 l/min (7% more than in group B) The MAP (see Methods) in group A was about 38% higher than in group B

In agreement with previous studies regarding hypertensives (7) the present study shows that there is a real relationship between body weight and BP However there is no straight forward explanation for this relationship as the hemodynamical differences related to the increased amount of adipose tissue are not great enough Hence there is a need to investigate further whether the relationship between BP and body weight may depend on hereditary or environmental factors

The women in group A had significantly higher B hematocrit fS triglyceride and S urate levels than those in group B It has been shown previously that obesity in males involves increased levels of B hematocrit fS triglycerides and S urate (1 11 16 24) Accordingly it was of interest to see whether significant differences between the groups remained after correction for the calculated influence of obesity The differences between the two groups did in fact disappear

The results further showed that the subjects in group A had higher heart rates than those in group B The same results have been demonstrated in males with borderline hypertension examined at the clinic (5) and in men with BPs above the 95th percentile (23)

The number of ST and T abnormalities was small but appeared to be more frequent in group A Caution is warranted when interpreting these results since Cumming et al (8) have demonstrated that ST T abnormalities are common in women especially during exercise and that these abnormalities in the majority of cases are not probably due to coronary artery disease The present study did not show any differences between the groups in R wave amplitude from a left ventricular lead (V_5)

The casual BPs measured at the time of selection and at follow up are not directly comparable as the statistical phenomenon of regression towards the mean (17) may have modified the results However the latter effect can hardly explain the increase in BP from the time of selection to 1972 in group A Moreover the large increase in BP could not be explained solely by the fact that the subjects were three years older at follow up a much smaller increase would have been expected (22) Psychological factors (13) may explain the differences in BP At the follow up in 1972 the females were aware that they had been selected for studies of their BPs and they had also been subjected to a rather intense campaign to recruit them into the study This may have made them more nervous before the examination in 1972 than at the time of selection When the subjects were reexamined in 1975 they were already acquainted with the examiner and with the procedures This could explain why their BP had decreased The rise in DBP and heart rate upon standing was of the same magnitude in the two groups and therefore did not separate them This is in agreement with earlier studies (15)

It has been recommended that patients should be allowed a significant period of rest (usually 5–10 min) before the BP is measured Although this recommendation seems easy to may involve significant trouble in a

Hence the question whether a casual BP measurement is acceptable or a resting BP level is required is not unimportant One criterion of a good BP measurement is that it does not vary from one examination to another due to factors which the doctor cannot control Judged from the present results the resting BP does not seem to be very much superior to the casual BP in this respect Accordingly, the assumed clinical superiority of the resting BP over the casual BP needs further validation

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Plasma Renin Activity, Aldosterone and Sodium Excretion in Women with High and Low Casual Blood Pressure Levels

Thomas Thulin Bengt E Karlberg and Bengt Schersten

From the Unit for Community Care Sciences Dalby and the Department of Internal Medicine University of Lund Lund University Hospital Lund and the Department of Internal Medicine and the Clinical Research Centre University Hospital Linkoping Sweden

ABSTRACT Sixty four women not under treatment for hypertension were selected from a population survey and examined with respect to the relationships of BP levels to plasma renin activity (PRA), plasma aldosterone as well as to the 24-hour urinary excretion of aldosterone, potassium and sodium under strictly defined conditions The subjects were selected from groups with BP levels above the 95th (group A) and below the 30th percentile (group B) The two groups were age matched PRA and plasma aldosterone were measured after one hour of complete rest in the recumbent position (basal PRA and basal plasma aldosterone), PRA was also measured after four hours of ambulation (upright PRA) as well as four hours after oral administration of 80 mg frusemide (stimulated PRA) The decline of PRA and of urinary aldosterone excretion with increasing age were related to the BP level but not to the menopause No correlations were found between PRA or plasma aldosterone on the one hand, and urinary sodium excretion on the other in women on random diets Therefore it is doubtful whether the correction of PRA levels for urinary sodium excretion is worthwhile unless marked sodium depletion or sodium loading is used Basal plasma aldosterone showed a high interindividual variability and was not correlated to age or BP

It has been proposed that the renin–angiotensin–aldosterone system has important diagnostic prognostic and therapeutic implications in hypertension (2 3 4 6 12 18) These studies have described a subgroup of patients with primary hypertension *characterized by low or unresponsive plasma renin* activity (PRA) Most of these patients have been females (8) often from the older age groups (27) However there is only a small number of studies in which the renin–angiotensin–aldosterone system has been evaluated systematically in relation to age (13 27) Moreover very few studies have been made in subjects recruited from an apparently healthy untreated population (7)

The present study concerns PRA aldosterone and sodium excretion in age matched groups of women with BP levels below the 30th and above the 95th percentile

Correspondence and requests for reprints to T Thulin M D The Unit for Community Care Sciences S-240 10 Dalby Sweden

STUDY POPULATION

The subjects were selected from participants in a general ophthalmologic survey (1) performed in Dalby community in 1969–70 BP was measured under standardized conditions (25) in 790 females not under treatment for hypertension

From this population a subset of 72 females 30–79 years of age was selected as described previously (24) Those with BPs above the 95th percentile constituted group A initially 36 females One subject refused to participate another failed to attend all investigations a third had been on antihypertensive treatment and a fourth had developed diabetes mellitus after the first examination in 1969–70 Thus group A finally consisted of 32 females For each individual in this group an age matched subject was chosen from the subjects in the population who had BPs below the 30th percentile (group B) The mean age (±S D) in 1975 in groups A and B was 52±14 and 52±13 years respectively

The two groups were further divided into four subgroups according to age in 1975 females up to 55 years of age (A_I $n=18$ and B_I $n=18$) and females 55 years of age or more (A_{II} $n=14$ and B_{II} $n=14$) None of the older

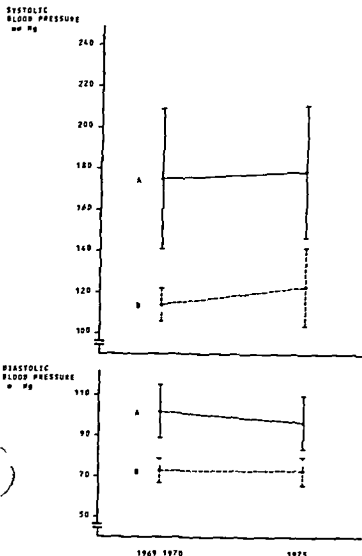


Fig 1 Casual systolic and diastolic BPs ($M \pm$ S D) in the two main groups *A* (—) and *B* ( ) in 1969–70 and 1975 (1 mmHg ≈0 133 kPa)

females menstruated The casual BPs in 1969–70 in the two main groups (A and B) are given in Fig 1

Except those mentioned above no subjects had any (other) signs of disease They had normal liver and kidney function as well as normal serum electrolytes (see Methods) None was on contraceptive pills or replacement estrogen therapy and they did not take any other drugs known to influence the parameters studied

METHODS

All investigations were performed during Jan –May 1975 The subjects fasted and had been requested not to smoke for at least ten hours before blood sampling

Measurement of blood pressure

The BP was measured as described previously (24) with a mercury manometer on the right arm before and after 10–15 min of rest in the recumbent position When the arm circumference was more than 30 cm an arm cuff with a rubber bladder measuring 15×43 cm was used otherwise the standard cuff with a rubber bladder of 12×35 cm Systolic BP (SBP) and diastolic (phase five) BP (DBP) were determined to the nearest 5 mmHg (1 mmHg≈0 133 kPa) All BPs were measured by one of the authors (T T) Heart rate (beats/min) was calculated from the radial pulse during 30 sec

Anthropometric and routine laboratory analyses

Height and weight were measured according to the WHO recommendations (22)

Creatinine potassium and sodium in serum and urine as well as liver function tests (S-ALAT S-ASAT and S-gammaglutamyltransferase) were determined by routine methods Kidney function was evaluated by means of the creatinine clearance and albumin excretion The creatinine clearance was related to m² BSA The urinary albumin was determined according to Mancini et al (20) and the 24-hour excretion rate was calculated

Plasma renin activity plasma and urinary aldosterone

In women with regular menstrual cycles the PRA and plasma aldosterone measurements were performed in the follicular phase (on day 5–10)

Blood for measurement of the PRA and plasma aldosterone was drawn according to the following time schedule 1) After one hour of complete rest in the recumbent position at 8–9 a m *basal PRA and basal plasma aldosterone* 2) After four hours of ambulation at 12 a m –1 p m *upright PRA* 3) Four hours after 80 mg frusemide (Lasix®) had been given orally under close supervision at 4–5 p m *stimulated PRA* The values of these three determinations constituted the PRA-frusemide test Venous blood for the PRA and plasma aldosterone determinations was collected in ice-cold EDTA Vacutainer tubes and immediately placed on ice Plasma was separated by centrifugation at +4 C for 15 min and then stored at −20°C until assayed

PRA was determined with a radioimmunoassay method measuring the amount of generated angiotensin I during optimal incubation conditions (pH 6 0) and with minimal dilution of plasma (10) The reference values ($M \pm$ S D) in our laboratory with this method with a sodium excretion rate of 70–220 mmoles/day in a normotensive population (n 82 age range 21–72 years) of both sexes were for basal PRA 0 43±0 27 upright PRA 0 85±0 55 stimulated PRA 1 61±1 12 pkat/l The coefficients of variation (CV) for this PRA method (for values within the normal range) were intra assay (n=100) 6 6% and inter assay (n=13) 7 7%

The plasma aldosterone and the 24-hour urinary excretion rate of aldosterone were determined by a direct radioimmunoassay method (21) using specific aldosterone antibodies (a gift from Dr J M McKenzie Winnipeg Canada) The reference value in our laboratory for basal plasma aldosterone with this method in a normotensive population (n=77 age range 21–72 years) of both sexes was 183±95 pmol/l (M ±S D) The CVs for the plasma aldosterone method were intra assay (n=215) 4 6% and inter assay (n=10) 11 5% Our reference range for urinary aldosterone in a normotensive population (n=117 age range 21–76 years) of both sexes was 11–70 nmol/l The CVs for the method were intra assay (n=127) 6 1% and inter assay (n=15) 10 5%

The 24-hour urine specimens were collected on the day before the PRA and plasma aldosterone measurements Precise instructions were given to each subject personally by one of the authors (T T)

Statistical methods

All the data were recorded on a previously prepared form and later transferred to punched cards and verified

Standard statistical methods were used to determine mean values standard deviations and the correlation coefficients The majority of the calculations were performed at the Computer Centre in Lund Statistical analyses were also made by using an Olivetti Programma 101 The hypothesis that no differences existed between groups A and B was checked by Wilcoxon s signed rank test This test was also used for evaluating intraindividual differences within a group Differences between two age groups within a certain main group were tested by Wilcoxon s rank sum test Spearman s rank-difference correlation coefficient was used throughout The significance levels were expressed as *0 01<p<0 05 **0 001<p<0 01 ***p<0 001 *The following symbols were used* N=number M=mean S D =standard deviation n s = not statistically significant (p>0 05) All values are given as M ± S D unless otherwise stated

RESULTS

The casual blood pressures in 1975 are shown in Fig 1 The mean values for age height weight BP and heart rate after rest in the different subgroups are given in Table I

Plasma renin activity

The mean PRA values (Table II) increased after stimulation (ambulation and frusemide) in all groups (p<0 001) The women in subgroup A_{II} had significantly lower levels of upright PRA and stimulated PRA than the women in subgroup B_{II} (p<0 05 and p<0 01 respectively) The women in subgroup A_{II} had significantly lower mean PRA values than those in subgroup A_I (p<0 05 p<0 01 and p<0 001 for basal upright and stimulated PRA respectively) No significant age related differences in PRA were found in group B

Table I *Age height weight systolic and diastolic BP after rest and heart rate after rest in the studied groups in 1975*

	Group A_I (N=18)	Group B_I (N=18)	p	Group A_{II} (N=14)	Group B_{II} (N=14)	p
Age (y)	42±7	42±7	n s	66±7	65±6	n s
Height (cm)	162±6	164±6	n s	157±4	160±6	n s
Weight (kg)	70 9±17 4	61 3±6 6		79 9±14 0	59 2±12 5	**
SBP (mmHg)	143±17	106±6	***	187±18	120±13	***
DBP (mmHg)	88±9	67±5	*	103±8	73±5	*
Heart rate (beats/min)	80±13	69±11		73±11	68±11	n s

Table II *Basal upright and stimulated PRA and basal plasma aldosterone values in the studied groups*

	Group A_I	Group B_I	p	Group A_{II}	Group B_{II}	p
PRA (pkat/l)						
Basal	0 44±0 29	0 39±0 25	n s	0 23±0 15[a]	0 34±0 26	n s
Upright	0 88±0 67	0 80±0 34	n s	0 38±0 28[b]	0 69±0 33	*
Stimulated	1 72±1 25	1 68±0 93	n s	0 57±0 52	1 50±0 89	**
Basal plasma aldosterone (pmol/l)	141±46	176±81	n s	114±52	169±82	n s

[a b c] Significant difference compared with group A_I ($p<0.05$ $p<0.01$ and $p<0.001$ respectively)

Table III *24 hour urinary aldosterone sodium and potassium excretions in the studied groups in connection with the PRA–frusemide test*

	Group A_I	Group B_I	p	Group A_{II}	Group B_{II}	p
Aldosterone (nmol/l)	31 6±12 9	23 3±13 5	*	15 7±6 3	15 6±5 4	n s
Sodium (mmol/24 h)	149±48	127±64	n s	143±62	122±40	n s
Potassium (mmol/24 h)	68±19	60±22	n s	50±14[b]	58±15	n s

[a b] Significant difference compared with group A_I ($p<0.001$ and $p<0.01$ respectively)

Plasma aldosterone

The mean plasma aldosterone values (Table II) did not differ statistically between the high and low blood pressure groups and were not influenced by ge in any of the groups

y aldosterone

mean values of the 24 hour urinary aldosterone excretion are given in Table III The urinary aldosterone excretion was significantly higher among those up to 55 years of age in group A than in group B ($p<0.05$) Among those 55 years of age or more there was no difference between groups A and B Subjects in subgroup A_{II} had a significantly lower urinary aldosterone excretion ($p<0.001$) than those in subgroup A_I In group B there was no age related difference in urinary aldosterone excretion

Urinary sodium and potassium

The 24 hour urinary sodium and potassium excretions (Table III) did not differ between the high and low BP groups and were not influenced by age Subjects in subgroup A_{II} had a significantly lower urinary potassium excretion ($p<0.01$) than those in subgroup A_I In group B there was no age related difference in urinary potassium excretion

Correlations between PRA urinary sodium excretion and urinary and plasma aldosterone

No correlations were found in any of the groups between PRA measured under the defined conditions and the 24 hour urinary excretion of aldosterone on the one hand and urinary sodium ex

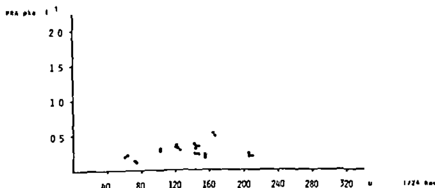


Fig 2 Basal PRA values and 24 hour urinary sodium excretion in groups A and B The correlation was not significant

cretion on the other Fig 2 shows the basal PRA values and 24 hour urinary sodium excretion for all subjects in the two groups There was a significant correlation between plasma aldosterone and urinary aldosterone excretion in group A ($r=0.67$ $p<0.001$) but not in group B ($r=0.31$ n s) Plasma aldosterone was not correlated to the urinary excretion of sodium Plasma aldosterone and basal PRA were found to be weakly correlated when the data from groups A and B were pooled ($r=0.31$ $p<0.05$)

DISCUSSION

The present study deals with PRA aldosterone and sodium excretion in apparently healthy women Most earlier studies have been performed in patients with hypertension (4 17) However Crane and Harris (7) investigated the effect of ageing on renin activity and aldosterone excretion in normotensive individuals They found a decline in PRA and urinary aldosterone excretion in the older subjects This decline appeared to be due to factors other than intake of sodium or potassium A decline in basal renin activity and its responsiveness to various stimuli with increasing age was found also in the present study However the PRA decline with age was restricted to the women with the high BP levels (group A) The decrease in PRA in older females might be secondary to a decrease in plasma renin substrate induced by the hypoestrogenemia of the menopause However if that was the case the decrease should have been the same in the two BP groups Further Crane and Harris did not observe a decline of renin substrate with increasing age

Another observation in the present investigation was that urinary aldosterone excretion decreased with advancing age in the high BP group and thus showed a pattern similar to the PRA Similar results have been reported by Flood et al (9) The diminished aldosterone excretion may be related to the lower renin secretion and decreased angiotensin II formation The urinary aldosterone excretion was significantly correlated to the basal plasma aldosterone level in the high BP group The basal plasma aldosterone values were not influenced by age in either group This may be explained by a high interindividual variability for plasma aldosterone The variability was not dependent on the radioimmunoassay method itself Indeed our inter assay variability was of the same magnitude as the plasma aldosterone assay described by Buhler et al (5)

The present findings concerning PRA aldosterone and sodium excretion do not support the concept proposed by Laragh s group (5 18 19) who stated that the basal plasma renin and aldosterone secretion move dynamically in inverse relation to the amount of urinary sodium excretion The discrepancy between their results and ours may be explained by the fact that their studies were performed on constant low (10 mmol/day) normal (100 mmol/day) or high (200 mmol/day) dietary sodium intakes for 4- or 5-day periods while our subjects were on random diets Hiner et al (14) who studied the PRA in relation to sodium excretion in children without dietary prescriptions have reported results similar to ours A rather strong sodium depletion (<40 mmol/24 hours) or sodium loading (>300 mmol/24 hours) is probably required to cause consistent changes in PRA (15)

It is possible that the older women in group A who could be expected to have been exposed to a high BP during many years and therefore also an increased renal perfusion pressure may have sustained a subclinical kidney damage in spite of normal kidney function tests The renin secretion has been shown to decrease with increasing renal perfusion pressure (16) Swales (23) has proposed that nephrosclerosis is associated with a diminished renin secretion and Gordon et al (11) have demonstrated that substantial kidney damage may be present in subjects with normal routine kidney function tests

The present study supports the assumption that there is a relationship in women between BP levels age and the activity of the renin–angiotensin–aldosterone system The results further support the suggestion that BP and age act synergistically in suppressing PRA This is in accordance with a recent study (26) It is doubtful whether it is worthwhile to correct the plasma renin levels for the urinary sodium excretion in subjects on random diets

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Effect of Upright Tilting on Kinins as Compared to Renin Activity in the Renal Venous Blood from Patients with Essential Hypertension

U L Hulthen H Lecerof and B Hokfelt

From the Departments of Endocrinology and Clinical Physiology University of Lund General Hospital Malmo Sweden

ABSTRACT The effect of tilting on the release of renal kallikrein as compared to renin was studied by the determination of kinin concentration and plasma renin activity (PRA) in the renal veins in supine position and after 15 min of 45 upright tilting in 10 patients with essential hypertension Kinin concentration decreased from 0 62±0 05 μg/l (mean± S E M) in supine position to 0 51±0 05 after tilting (p<0 01), while PRA increased from 2 84±0 39 μg/l/3 h to 4 87±0 66 (p<0 001) These results indicate that tilting diminishes the release of renal kallikrein It is suggested that decreased intrarenal generation of kinins may be of importance for the reduction of diuresis and natriuresis induced by tilting

Kinins are generated by kallikreins (23) They increase renal blood flow diuresis and natriuresis in man (1) Upright tilting causes a reduction of renal blood flow diuresis and natriuresis in normotensive and hypertensive subjects (17 18) It was thus considered of interest to investigate the effect of tilting on renal kinin production

Renin secretion is closely related to salt water homeostasis and is also under orthostatic influence (3 4) We have therefore determined plasma renin activity (PRA) simultaneously with kinins in renal venous blood in order to compare the effect of tilting on the renal release of kallikrein and renin PRA was also determined in renal and peripheral veins for diagnostic purposes

PATIENTS AND METHODS

The studies were performed in ten patients with essential hypertension (Table I) One patient (no 6) also had acromegaly All patients had hypertension in stage I–II according to WHO classification Isotope renogram was normal in all patients Renal angiography was performed in four patients and showed minor functionally non-significant irregularities in the renal arterial wall on one side in three cases and on both sides in one case There was no difference between PRA in the right and the left renal vein PRA in peripheral vein was comparatively high in six patients when measured in supine position and urinary excretion of aldosterone was above the normal range in five (Table I) In the remaining patients PRA and aldosterone were within the normal range All patients had normal values for urinary catecholamines

The patients had been off all medication for at least one week They were kept in bed and fasted overnight prior to catheterization The procedure for renal vein catheterization has been described earlier (9) A polyethene catheter PE 160 (Intramedic® Clay Adams New York) was also inserted into an antecubital vein and advanced about 20 cm in cephalic direction Samples for the determination of kinins and PRA were drawn simultaneously from the renal veins and immediately thereafter from the brachial vein for the assay of PRA firstly in supine position and then after 15 min of 45 upright tilting Patient 5 experienced vasovagal symptoms and samples therefore had to be drawn already after tilting for 8 min Kinin concentration was not assayed in brachial venous blood because we have found that an indwelling catheter in peripheral veins often activates blood kallikrein (unpublished observation)

Blood kinins were determined by radioimmunoassay (8) For each patient all samples taken were measured in one and the same single assay run The intra assay coefficient of variation for the means of triplicates was 12 6%

PRA was measured as the amount of angiotensin I generated following three hours incubation (7) Again all samples from each patient were measured in one and the same single assay run The intra assay coefficient of variation for the mean of duplicates was 10%

Urinary catecholamines were measured by a fluorometric procedure (6) and urinary aldosterone by a modification of the radioimmunoassay published by Ito et al (10)

Statistical significance for changes in kinin concentration and PRA was assessed by Student s t test for pairs of dependent samples

RESULTS

The mean kinin concentration in the renal veins decreased from 0 62±0 05 μg/l (mean ± S E M) in

Table I *Basal clinical data peripheral plasma renin activity (PRA) and urinary aldosterone excretion in the patients studied*

Pat no	Sex	Age (y)	Supine BP	Endogenous creatinine clearance (ml/min/1 73 m²)	I v pyelography[a]	Renal angiography	Supine PRA (μg/l/3 h)[b]	Urinary aldosterone (nM/24 h)[c]
1	♀	39	150/100	110	N	–	2 84	100 0
2	♀	43	170/105	118	N	–	0 53	30 0
3	♀	45	190/115	154	–	Minimal stenosis in left renal artery	3 91	53 9
4	♀	47	190/105	82	N	Minimal stenosis in both renal arteries	1 62	8 1
5	♀	55	180/100	86	N	–	3 56	56 6
6	♂	39	170/110	117	N	–	3 14	54 0
7	♂	44	140/100	143	N	–	1 61	24 4
8	♂	45	180/105	138	–	Minimal stenosis in left renal artery	3 78	36 1
9	♂	46	150/100	87	N	–	2 58	55 8
10	♂	51	180/110	106	–	Minimal stenosis in right renal artery	1 22	9 0

[a] N=normal [b] Normal range 0 5–2 0 [c] Normal range 8–40

supine position to 0 51±0 05 after tilting ($p<0\ 01$) (Table II)

PRA in renal veins increased from 2 84±0 39 μg/l/3 h (mean ± S E M) in supine position to ±0 66 after tilting ($p<0\ 001$) (Table III) In vein PRA increased from 2 14±0 40 in position to 3 29±0 74 after tilting ($p<0\ 025$) the renal veins PRA was 31±16% (mean ± S D) higher than in peripheral vein in supine position and this difference increased to 52±16% after tilting This increase in the ratio of PRA in renal veins to PRA in peripheral vein produced by tilting was statistically significant ($p<0\ 001$) None of the patients showed any significant difference between PRA in the right and the left renal vein neither in supine position nor after tilting the individual ratios ranging 86–117%

Table II *Kinin concentrations (μg/l) in right (R) and left (L) renal vein in supine position and after tilting*

Pat no	Supine R	Supine L	Tilted R	Tilted L
1	0 73	0 59	0 67	0 75
2	0 62	0 73	0 51	0 46
3	0 30	0 49	0 24	0 47
4	1 14	0 64	0 68	0 48
5	0 60	0 92	0 60	0 66
6	1 08	0 68	1 12	0 66
7	0 59	0 58	0 32	0 39
8	0 41	0 36	0 40	0 33
9	0 71	0 64	0 55	0 41
10	0 31	0 21	0 27	0 29
Average (mean ± S E M)	0 62±0 05		0 51±0 05	

DISCUSSION

As discussed in a previous paper (9) studies in dogs give good evidence that changes in renal venous levels of kinins reflect changes in renal kallikrein activity (15 19) It is thus most likely that the present demonstration of a decrease in kinin levels in *the renal vein after tilting reflects diminished release* of renal kallikrein

In patients with essential hypertension 45° of upright tilting significantly reduces the mean renal blood flow (17) This might mask a decrease in renal kinin generation when measured as kinin concentration in the renal veins The decrease in renal kallikrein release after tilting thus has to be considerable in order to significantly decrease kinin concentration in the renal veins as found in our studies

The stimulus leading to diminished release of renal kallikrein after tilting may be the reduction in renal blood flow In rats administration of furosemide bumetanide or hydralazine which increases renal blood flow has been found to enhance urinary excretion of kallikrein (5 11) which is of

Table III *Plasma renin activity (µg/l/3 h) in right renal vein (R) left renal vein (L) and peripheral vein (P) in supine position and after tilting*

Pat no	Supine R	L	P	R/P (%)	L/P (%)	R/L (%)	Tilted R	L	P	R/P (%)	L/P (%)	R/L (%)
1	1 71	1 89	1 27	135	149	90	3 40	3 26	2 07	164	157	104
2	1 45	1 48	1 06	137	140	98	1 46	1 33	1 00	146	133	110
3	6 49	6 99	4 80	135	146	93	12 11	12 62	9 42	129	134	96
4	2 72	2 42	1 72	158	141	112	4 26	3 73	2 30	185	162	114
5	4 17	4 03	3 47	120	116	103	5 95	5 92	3 74	159	158	101
6	3 95	4 00	3 00	132	133	99	6 14	5 94	3 74	164	159	103
7	1 11	1 22	1 13	98	108	91	3 23	3 23	2 37	136	136	100
8	1 83	1 87	1 62	113	115	98	4 14	4 79	3 00	138	160	86
9	3 73	3 56	2 45	152	145	105	5 04	5 47	3 61	140	152	92
10	1 11	1 08	0 92	121	117	103	2 89	2 46	1 60	181	154	117
Average	2 84±0 39[a]		2 14±0 40[a]	131±16[b]			4 87±0 66		3 29±0 74[a]	152±16[b]		

Mean ± S E M [b] Mean ± S D

renal origin (20) In dogs a decrease in renal blood flow following constriction of the renal artery reduced urinary kallikrein excretion (2 12) In man the venous output of kinins was found to be lower from the kidney with pronounced artery stenosis than from the kidney with non stenotic artery (9) Recently a significant positive correlation was reported between urinary kallikrein activity and renal blood flow in man (13)

A decrease in the intrarenal generation of kinins may be of importance for the reduction of urinary excretion of water and sodium observed after tilting In dogs inhibition of kininase II was associated with renal vasodilatation diuresis and natriuresis (19) and rats treated with antibradykinin serum showed reduced ability to excrete an i v saline load (14)

Peripheral venous kinin levels increased in normal man on standing (22 24) As this increase was not accompanied by significant changes of prekallikrein kallikrein inhibitor or kininase in peripheral plasma Wong et al (24) speculated that increased kinin levels in peripheral vein reflected increased renal kallikrein release Our results do not support this hypothesis

The mean difference of 31% between PRA in the renal veins and peripheral vein in supine position is very similar to the figures reported by Maxwell et al (16) and also Sealey et al (21) in patients with essential hypertension The greater mean difference (52%) found after tilting was probably due to the reduction of renal blood flow The standard deviation of this mean difference was identical to that in supine position despite the lack of steady state conditions after only 15 min of upright tilting Thus the interindividual variation for the reduction of renal blood flow after tilting was small

Our results indicate that tilting decreases the release of renal kallikrein It seems possible that decreased intrarenal generation of kinins is of importance for the reduced diuresis and natriuresis observed after tilting

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Acute and Long-Term Salt Depletion and β-blockade Plasma Renin Activity Response and its Relation to Blood Pressure Reduction in Long-Term Treatment

Ingolf Nielsen Eva Steiness and Birger Hesse

From Medical Department B Rigshospitalet and the Department of Pharmacology University of Copenhagen Copenhagen Denmark

ABSTRACT The changes in plasma renin activity (PRA) during short term salt depletion (and peroral furosemide on the first day) and after bolus injection of propranolol were compared to the change during long term treatment with diuretic and with propranolol in 19 patients with benign primary hypertension A highly significant correlation was found between PRA on short term and long term salt depletion ($r=0\ 92$) A highly significant correlation was likewise found between initial PRA and decrement of PRA after bolus injection of or long term treatment with propranolol Only a weak inverse correlation was found between PRA reached during short term salt depletion or long term diuretic treatment and the fall in diastolic BP during long term treatment ($r=-0\ 60$) No significant correlation was found between decrease in PRA on propranolol (bolus/long term) and diastolic BP reduction It is concluded that the short term PRA response to salt depletion and propranolol in the individual patient gives a good prediction of the PRA level on long term diuretic or propranolol treatment but is of no value in predicting the BP reduction during treatment

Beta blockers and diuretics are in widespread use in the treatment of hypertension due to their efficacy and high degree of freedom from side-effects However in a seemingly homogeneous group of primary hypertensives there is a great variability in the BP response to the agents The observation of Buhler et al (2) that high renin hypertensives responded best to β-blocker (propranolol) treatment and the observation of Douglas et al (4) that low renin hypertensives responded best to diuretics (chlorothiazide) raised the hope that renin measurements could be used for the selection of patients for the most effective therapy Recent communications have not unanimously been able to confirm the original observations In agreement with Laragh s group (2) are Shand et al (14) and Karlberg et al (9) but several investigators (1 3 8 10 11 16) were unable to confirm the original observation The finding of Douglas et al (4) was confirmed by Karlberg et al (9) but not by Dorhout Mees et al (3) or Woods et al (16)

All the previous investigations have dealt with long term observations In the present work acute and long term effects of salt depletion and propranolol on plasma renin activity have been compared and related to BP reduction on long term treatment with diuretic and propranolol

PATIENTS

Nineteen patients four women and 15 men (average age 45 years range 23–62) with primary hypertension according to WHO criteria I and 2 were examined None of the patients had previously received antihypertensive treatment Before admission the patients were seen 3–4 times at weekly intervals The BP and plasma renin activity (PRA) (at 2–4 p m 45 min supine) on the two last visits were averaged and formed the control values The range of diastolic BP was 110–140 mmHg and the range of PRA 0 5–12 1 ng/ml/h

Protocols

Four days salt depletion On the second day of admission nine patients were put on a salt poor balanced diet (10 mmol Na 70 mmol K^+) for four days On the first day 80 mg furosemide (Lasix®) was given perorally Peripheral vein PRA was measured on the first morning (at 9 a m after overnight recumbency and fasting) and on the fifth morning BP fell in most cases the range of the diastolic BP fall being 0–15 mmHg

Table I *Diastolic BP (DBP) and plasma renin activity (PRA) before and during treatment with bendroflumethiazide propranolol and a combination of the two (mean ± S E M)*

	DBP (mmHg)	PRA (ng/ml×h)
Control	119±2 0	3 5±1 0
Diuretic	109±3 0	6 4±1 3
Propranolol	105±3 0	1 7±0 2
Propranolol + diuretic	99±3 0	3 5±1 1

Injection of propranolol Ten patients without prior treatment and four on bendroflumethiazide (Centyl®) treatment for approximately three months (patients already in randomized treatment—see below) At 9 a m on the second day of admission after overnight recumbency and fasting 10 mg propranolol (Inderal®) was given into an indwelling needle in an antecubital vein and blood samples for PRA and plasma propranolol concentration were drawn before propranolol and 5 10 15 30 45 60 120 180 and 240 min after injection BP was measured at close intervals no change was observed

Randomized treatment At discharge the patients were randomly started on either bendroflumethiazide 10 mg b i d or propranolol 80 mg t i d or both The patients were seen every 4 weeks in the Out Patient Clinic always the afternoon (at 2–4 p m) BP PRA and plasma concentration were measured at each visit approximately 12 weeks the therapy was changed to of the other two regimens and after a further 12 weeks the last regimen was instituted Five patients with satisfactory BP response to a one-drug regimen were not put on combined therapy but always on the other one drug regimen Eight patients left the trial for non medical reasons before completing the third phase

METHODS

PRA was measured by radioimmunoassay using the NEN kit (angiotensin I–^{125}I Haber method coefficient of variation=8 2% (day to-day determination)) Normal range of PRA in our laboratory is 0 7–2 9 ng/ml/h Plasma propranolol concentration was measured by a gas chromatographic method previously described by Walle (15)

Wilcoxon s test for paired samples was used for determining the significance of differences

RESULTS

After treatment for four weeks diastolic BP (DBP) and PRA showed values which did not deviate significantly from the next monthly determinations Accordingly the effect of the drug was sustained Hence in the following all values are averages from the three determinations on each regimen

Diuretic treatment (Table I) A significant fall in DBP ($p<0\ 001$) and a significant increase in PRA ($p<0\ 01$) were found

There was a significant correlation between PRA after four days of salt depletion and PRA on long term diuretic treatment ($r=0\ 92$ $p<0\ 001$ $n=10$) (Fig 1)

There was a significant correlation between control PRA and decrement in DBP ($r=-0\ 57$ $p<0\ 05$ $n=14$) and between PRA on long term diuretic treatment and decrement in DBP ($r=-0\ 62$ $p<0\ 02$ $n=14$)

Propranolol treatment (Table I) A significant fall in DBP ($p<0\ 01$) and a significant fall in PRA ($p<0\ 01$) were found The fall in DBP was not significantly different from the fall during diuretic treatment ($p>0\ 10$)

In the propranolol injection test PRA fell in all cases within the first hour and had regained the initial value in three hours The higher the initial PRA value the deeper the fall in PRA (Fig 2) The same pattern was seen in long term treatment The paired values (initial value PRA maximal decrement PRA) for acute and sustained propranolol medication showed a significant correlation ($r=0\ 90$ $p<0\ 001$ $n=29$ regression line $y=0\ 60x-0\ 66$) (Fig 2) If the mean of the initial and final values is used instead of the initial value (a statistically more correct approach) the correlation coefficient is unchanged

The correlation between control value of PRA and long term decrement of DBP was not significant ($r=0\ 42$ $p>0\ 10$ $n=15$) neither was the correlation between long term decrement of PRA and long term decrement of DBP ($r=0\ 41$ $p>0\ 10$ $n=15$)

After i v injection of propranolol high initial

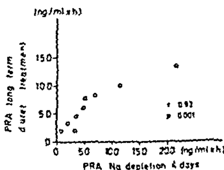


Fig 1 Correlation between PRA after four days of salt depletion and PRA on long term diuretic treatment

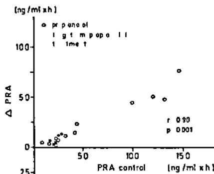


Fig 2 Correlation between control PRA and PRA reduction after bolus injection of propranolol and on long term propranolol treatment

plasma concentrations were reached after which a gradual decline was observed During the distribution phase the individual variations were large while they became very small during the later elimination phase (Fig 3) During long term treatment the range of plasma propranolol concentration was 40–200 ng/ml No correlation was found between propranolol concentration and decrement in DBP or decrement in PRA (r=0 35 and 0 20 respectively p>0 10 n=15)

Combined treatment with propranolol and diuretic The DBP fell more than on either single drug regimen (p<0 02) PRA on combined treatment was not significantly different from the control values (p>0 10)

There was a significant correlation between PRA on diuretic treatment and decrement in PRA when propranolol was added (r=0 87 p<0 001 n=10 regression line y=0 65x−0 87)

DISCUSSION

In the group as a whole it appears that propranolol bendroflumethiazide and the combination of the two drugs have a sizeable antihypertensive effect the former two being of almost equal potency the combination giving the additive effect of the single drugs However it is evident that the range of antihypertensive response from patient to patient is pronounced This well known observation underlines the usefulness of establishing tests that predict the efficacy of β blockade and diuretic in the individual patient

Diuretic treatment gave a sustained increase in PRA which was inhibited by the addition of propranolol Bravo et al (1) and Omvik et al (12) did not find a sustained PRA reducing effect of propranolol in the same setting

In diuretic treatment a significant but rather weak correlation was found between the PRA level reached on treatment and the DBP reduction attained This is in general agreement with findings of Douglas et al (4) and Karlberg et al (9) There was a highly significant correlation between the PRA level on long term treatment with diuretic and the level reached at four days salt depletion with furosemide added Hence this test could be suggested as a means of selecting patients for diuretic treatment But since the correlation between control PRA and DBP reduction during long term diuretic treatment was almost as strong it seems hardly worthwhile to perform the test The r value of the regression indicates that only 36% (r^2=(0 6)^2=0 36) of the variation in DBP reduction is explained by PRA variation illustrating the weak power of prediction from PRA measurements before treatment

In propranolol treatment no correlation was found between initial PRA or PRA decrement during the treatment and DBP reduction Accordingly PRA measurements could not be used to predict the efficacy of propranolol treatment These results are in agreement with previous findings of several groups (1 3 8 10 11 16) There was a highly significant correlation between control PRA level and decrement in PRA influenced by propranolol comprising both short term and long term medication In other words the percentual PRA reduction was the same irrespective of the initial PRA level (about 60% of control level the slope of the regression line being 0 6)

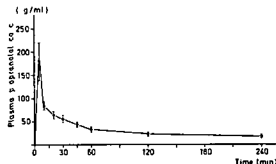


Fig 3 Plasma propranolol concentration (mean±S E M) in 14 patients after i v bolus injection of 10 mg propranolol

At the plasma propranolol concentration level reached in long term treatment complete β blockade can probably be assumed (1 13) Accordingly the fraction of PRA level due to sympathetic tone should be removed The homogeneous PRA reduction to approximately 60% of the initial level argues against any great variability of sympathetic PRA stimulation in benign primary hypertension

In combination treatment with propranolol and bendroflumethiazide an additive antihypertensive effect was reached However it was not so that patients who attained a high decremental PRA reduction during addition of propranolol responded with a specially pronounced DBP reduction during combination therapy In combination treatment PRA level was reduced to 65% of the initial level (PRA level on long term diuretic) (the slope of the regression line being 0 65) This reduction is approximately the same as in propranolol treatment alone (60%) indicating the same proportion of sympathetic PRA stimulation during diuretic treatment While PRA reduction on propranolol treatment probably indicates the state of sympathetic involvement in PRA stimulation it is obviously a or measure of the role of the renin system as such BP homeostasis Measurement of BP reduction injection of saralasin might well be better suited in this respect (5 6)

In conclusion a short term salt depletion test is well suited to predict the PRA level on long term diuretic treatment but PRA response is a poor predictor of antihypertensive efficacy of diuretic treatment Propranolol given in a bolus or as sustained medication will by PRA reduction indicate the proportional involvement of the sympathetic system in PRA stimulation The role of renin in keeping up the BP level is not established by the propranolol bolus test or sustained propranolol medication and propranolol medication of short duration will not predict the antihypertensive efficacy of long term treatment

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Changes in Plasma Volume and Extracellular Fluid Volume after Addition of Hydralazine to Propranolol Treatment in Patients with Hypertension

H Ibsen K Rasmussen H Ærenlund Jensen and A Leth

From the Department of Clinical Physiology and Department of Medicine C Glostrup Hospital and Department of Medicine C Diakonissestiftelsen Copenhagen Denmark

ABSTRACT In 16 patients with hypertension BP could not be controlled satisfactorily by treatment with propranolol alone (mean dosage 325 mg/day) Plasma volume (PV) (T 1824) and extracellular fluid volume (ECV) (^{82}Br-distribution space) were determined in these patients before and after the addition of hydralazine for three months (mean dosage 135 mg/day) After the addition of hydralazine, PV and ECV increased significantly, by 9% and 3%, respectively Systolic and diastolic BPs decreased by 15% and 13% The mechanisms inducing fluid retention during treatment with hydralazine and the clinical significance of the problem are discussed It is concluded that the addition of a diuretic to propranolol–hydralazine treatment is often well indicated

In most patients with established arterial hypertension *the total peripheral vascular resistance is* abnormally high (6) Hence on theoretical grounds it would seem rational to include direct acting peripheral vasodilators in the treatment of hypertension A reduction in arteriolar tone leads to an activation of the baroreceptors resulting in a marked rise in cardiac output and heart rate (3 7 16 29) The reflex cardiac stimulation not only impairs the antihypertensive effect of the drug (16) but may also lead to unacceptable side-effects such as palpitations tachycardia and angina Furthermore headache and flushing limit the clinical use of hydralazine As β blocking agents inhibit the cardiac stimulation and minimize the troublesome side effects arising from vasodilators (7 9 20 31) the combination of hydralazine and β blockers has proved to be a very useful antihypertensive treatment as documented in many studies (7 9 11 20 29 31)

It has been maintained that most potent antihypertensive drugs are likely to cause fluid retention thereby leading to expansion of plasma volume (PV) and extracellular fluid volume (ECV) diminishing the effect of the treatment (4 5 22) The influence of β blockers on PV and ECV is modest PV remains virtually unchanged (14 24) or slightly reduced (25) On the other hand we found a small but significant 5% increase in ECV during propranolol treatment (24) Several investigations have revealed an increase in PV and ECV caused by peripheral vasodilators This applies mostly to diazoxide (5) and minoxidil (7 29) while information about the influence of hydralazine on body fluid compartments is still sparse (5 9)

The object of the present study was to investigate body fluid compartments after the addition of hydralazine to the antihypertensive treatment in patients not successfully controlled on propranolol alone

PATIENTS AND METHODS

The group studied comprised 16 patients (13 men and 3 women) with hypertension of medium severity They had not achieved a satisfactory BP level during treatment with propranolol

An examination program previously described (12) served to exclude or define recognized etiologies of hypertension Fifteen patients had essential hypertension and in one (no 12) the hypertension was caused by

Table I *Clinical data on 16 patients with hypertension during antihypertensive treatment with propranolol*

Pat no	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m²)	BP (mmHg)	Serum creatinine (mg/ 100 ml)	⁵¹Cr EDTA clearance (ml/min 1 73 m²)	Plasma volume (ml)	Extracellular fluid volume (ml)
1	♀	40	154	60 0	1 58	192/102	7	99	2 239	12 046
2	♀	40	160	59 0	1 61	188/120	8	78	1 821	11 793
3	♂	22	172	54 8	1 64	165/110	10	79	2 138	13 312
4	♂	62	167	60 2	1 67	180/115	14	42	2 224	13 355
5	♂	60	172	61 5	1 71	181/106	12	72	2 973	15 490
6	♀	47	168	67 0	1 76	220/140	10	84	2 901	14 812
7	♂	47	178	65 0	1 81	158/98	12	64	2 650	14 204
8	♂	33	170	77 5	1 89	153/108	10	98	2 152	14 427
9	♂	44	177	72 8	1 89	188/125	10	72	2 075	13 517
10	♂	47	176	77 5	1 94	190/120	16	56	3 061	17 148
11	♂	48	167	89 7	1 99	198/118	11	54	2 819	16 507
12	♂	38	180	81 4	2 01	185/121	14	60	2 778	16 903
13	♂	68	172	89 3	2 02	167/110	11	–	2 871	18 875
14	♂	54	183	80 5	2 03	188/110	20	35	3 594	21 432
15	♂	54	169	103 2	2 12	198/112	11	67	3 112	20 547
16	♂	62	185 5	88 1	2 13	161/108	14	57	3 619	20 650

chronic glomerulonephritis All had normal heart size determined by chest X ray and none had signs of congestive heart failure Renal function was assessed from creatinine and at the initiation of the study by ination of ⁵¹Cr EDTA clearance (2) Six patients moderately to severely decreased kidney function included in the study as the plasma creatinine concentration of these patients had been at a constant level for at least one year All patients presented retinal changes corresponding to grade 1 or 2 (Keith Wagener & Barker scale)

All patients had been treated with a constant dosage of propranolol 160–640 mg/day (mean 325) for at least three months On this treatment the diastolic BP was 100 mmHg or more at the latest 2–3 check ups in the Out Patient Clinic Clinical data from this period are given in Table I

All examinations were carried out at 9 a m The patients had had nothing to eat or drink for 9 hours and had rested in bed for one hour and during the examinations they were strictly confined to bed All investigations were undertaken in the Out Patient Clinic No dietary restrictions were imposed but the patients were instructed to avoid major changes in their usual diet The aim of the study was explained to the patients and their consent was obtained

Before the addition of hydralazine while the patients were on propranolol exclusively PV and ECV were determined as previously described (17) PV was measured with T 1824 (Evans blue) The amount of dye administered was determined gravimetrically by weighing the syringe before and after injection The theoretical plasma extinction D_o at time $t=0$ was found by linear regression from a semilogarithmic graph by the method of least squares using the extinctions at $t=15$ 30 45 and 60 min ECV was determined as the distribution volume of ⁸²Br About 15 μCi of ⁸²Br⁻(NH₄ ⁸²Br) was injected i v Following an equilibration period of 4 hours peripheral venous blood samples were taken for determination of radioactivity Individual correction for loss of radioactivity to erythrocytes and urine was employed In calculating the distribution space for ⁸²Br a fixed correction factor of 0 93 was used for plasma protein and Gibbs Donnan effect (17)

All injections were given through the membrane of an indwelling needle and venous blood samples were drawn from an antecubital vein in the contralateral arm The body weight was registered and the BP measured after one hour's rest in supine position A standard arm cuff and a mercury manometer were used The diastolic BP was determined at the disappearance of the Korotkoff sounds (phase V) The mean of three measurements was calculated After the initial procedures the propranolol treatment was combined with hydralazine and at check ups in the Out Patient Clinic the dosage was increased until a satisfactory BP level was obtained or a maximal dosage of 200 mg/day was reached Propranolol dosage was kept unchanged during the whole period At the check ups BP was measured after 15 min rest in supine position For assessment of changes in BP the measurements in the Out Patient Clinic were used Mean arterial pressure (MAP) was calculated as the diastolic BP plus one third of the amplitude After combined treatment with hydralazine in a fixed dosage of 37 5–200 mg/day (mean 135) for three months the parameters mentioned above were again determined Hydralazine (Apresolin®) was placed at our disposal by Ciba-Geigy

Changes in the parameters investigated are given in absolute figures and as percentage changes with reference to initial measurements For the testing of zero-hypotheses Wilcoxon s test for paired differences and Student s paired *t* test were applied

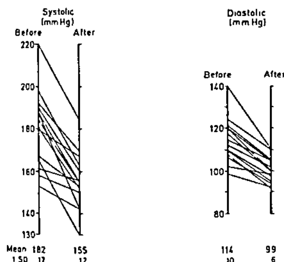


Fig 1 Changes in BP after addition of hydralazine to propranolol treatment in patients with hypertension

RESULTS

Addition of hydralazine to the treatment brought about a marked reduction in systolic as well as diastolic BP on an average by 27 mmHg (S D ±17) and 15 mmHg (S D ±6) respectively As shown in Fig 1 not all patients obtained a satisfactory BP level In 6 patients the diastolic BP remained above 100 mmHg

Changes in PV and ECV are presented in Fig 2 A significant ($p<0\,01$) mean increase in PV of 223 ml (S D ±219) equal to 8 7% was found Changes in ECV were less pronounced The mean increase was 552 ml (S D ±758) equal to 3 3% ($p<0\,05$) Changes in body weight were insignificant and did not correlate to changes in PV or ECV

There was no correlation between changes in PV or ECV and changes in BP neither diastolic nor calculated MAP However 5 out of 6 patients with diastolic BP remaining above 100 mmHg belonged to the group with the largest increases in PV (more than 9%) whereas only 2 out of 10 patients with satisfactory BP control were found in this group ($p<0\,05$ Fisher s exact test)

DISCUSSION

The present investigation revealed significant increases in PV and ECV by about 9% and 3% respectively when hydralazine was added to the treatment of patients who were not successfully controlled on propranolol alone

It has often been maintained that hydralazine like other peripheral vasodilators induces sodium retention and expansion of PV and ECV However direct measurements of body fluid compartments during hydralazine treatment have been rather scarce In acute and short term experiments hydralazine may increase renal blood flow and sodium excretion (8 15) provided that the fall in BP is only modest (8) With a marked reduction in BP sodium excretion decreases (8 18 32)

In 5 patients who had developed drug resistance to hydralazine Finnerty et al (5) found a marked rise in ECV and PV on an average 1 86 and 0 44 l respectively During treatment with new more potent vasodilators very pronounced sodium retention has been reported (5 7 29) In 4 patients receiving daily injections of diazoxide Finnerty et al found an increase in ECV of 3 27 l after 10 days treatment and at the same time the antihypertensive effect was blunted Minoxidil in combination with propranolol also gives rise to sodium retention and PV expansion (7) In studies including patients with severely decreased renal function increasing dosage of loop diuretics was required to control sodium retention (29) A comparative study of minoxidil and hydralazine in combination with propranolol and hydrochlorothiazide (9) disclosed a tendency to sodium retention during the minoxidil treatment occasionally demanding an intensified

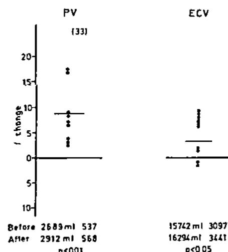


Fig 2 Changes in plasma volume (*PV*) and extracellular fluid volume (*ECV*) after addition of hydralazine to propranolol treatment in patients with hypertension (mean + S D)

diuretic medication However this indication of fluid retention was not seen in connection with hydralazine treatment despite a very high dosage of the drug We did not include a diuretic in our study and this explains the seeming discrepancy in results between our investigation and the study last mentioned Our preliminary experience with prazosin also showed expansion of PV and ECV when this new vasodilator was added to propranolol (13) We are not aware of ohter investigations elucidating the influence of hydralazine–β blocker treatment on body fluid compartments

The mechanisms behind sodium retention brought about by vasodilators are not fully clarified Experimental studies have shown that hypertensive kidneys require an abnormally high perfusion pressure to excrete normal amounts of sodium, and if perfused at a normal pressure they will excrete subnormal amounts (26 27) This would lead to sodium retention and a tendency to maintain hypertension This might well be a major responsible factor shared by virtually all antihypertensive drugs except for diuretics Other experimental data suggest that vasodilators in another possibly more specific way alter the intrarenal distribution of flow leading to an enhanced sodium re in the proximal renal tubule (32) A contributing factor could be an effect mediated via the adrenals However sodium retention caused by hydralazine was unaffected by bilateral adrenalectomy in rats (10) Furthermore in man it has been found that plasma aldosterone and aldosterone excretion were only slightly increased (7) or unchanged (9) during treatment with minoxidil or hydralazine

Changes in body weight were inconsistent In accordance with our previous studies we must conclude that body weight as a measure of changes in fluid volume during long term studies must be interpreted with caution being a very crude measure Several feed back mechanisms interfere with the hypotensive potency of vasodilators As mentioned before the fall in peripheral vascular resistance leads via the baroreceptors to an activation of autonomic reflexes resulting in increased cardiac output and thus limiting the hypotensive response (16 30) Hydralazine like several other vasodilators except prazosin raises the plasma renin level (9) and thereby angiotensin II Studies with angiotensin II inhibitor (Saralasin) (21) have revealed that the activation of the renin–angiotensin system counteracts the fall in BP β Blocking agents not only inhibit the increased sympathetic activity arising from the use of vasodilators (7 9 31) but also block the augmented renin release (9 19 21) There is indeed evidence that in this situation the antihypertensive mechanism of propranolol to some extent is caused by its renin suppressing effect (21) Thus from a theoretical point of view it seems rational to combine vasodilators and β blockers A third mechanism limiting the hypotensive effect is the expansion of PV and ECV as demonstrated in the present and other studies (4 5 7) It has been pointed out in several studies that resistance to antihypertensive drugs is related to fluid retention and the use of diuretics will restore the sensitivity to the treatment (4 5 22) It has also been found that acute expansion of ECV blunts the hypotensive response to i v administered diazoxide hydralazine and reserpine (5) We did not find a close relation between changes in BP and changes in fluid volumes but 5 out of 6 patients with unsatisfactory BP control showed the greatest increase in PV

Thus it can be concluded that it is well indicated to include a diuretic in the treatment when the combination of vasodilator and β blocker does not lead to a proper BP control Our preliminary results with measurements of fluid volumes and BP after the addition of hydrochlorothiazide (13) favour this concept

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Adverse Reactions with Methyldopa—a Decade's Reports

Anna Karin Furhoff

From the Section of Adverse Drug Reactions Department of Drugs National Board of Health and Welfare Uppsala Sweden

ABSTRACT During 1966–75 the Swedish Adverse Drug Reaction Committee received some 300 reports of reactions to methyldopa As the Swedish rules for reporting adverse reactions emphasize the importance of severe and unexpected reactions the relative incidence of various adverse effects differs greatly from what would be found in an intensive study The three most commonly reported reactions are fever hemolysis and hepatic effects Two thirds of these patients were women The median age of the groups differed from 57 years (hepatic effects) to 69 years (hemolysis) The clinical picture of the different reactions was consistent in the main with what has been described previously Between 70 and 85% of the patients in these groups were admitted to hospital because of the adverse reaction

Methyldopa has been used in Sweden for treatment of hypertension since the beginning of the 60s In the period 1971–75 methyldopa sales varied between 11 2 and 16 8 tons a year corresponding to 3 7–5 7 defined daily doses per 1000 inhabitants (one defined daily dose=1 g 1 methyldopa) (5)

Adverse reactions of various kinds have been reported over the years Tiredness sleepiness nasal obstruction and dryness of the mouth occur chiefly at the beginning of treatment and are then often transient Nightmares depression paresthesias and Parkinsonism have been reported Sexual disturbances are probably not uncommon There have also been reports of altered intestinal function (flatulence diarrhea obstipation) edema with weight increase and galactorrhea Fever is reported to occur in about 3% and is considered to have an allergic genesis as is the hepatic reaction which may likewise occur with methyldopa treatment Hemolytic anemia is another potentially serious reaction with an immunologic background (8) Although deaths have been reported in connection with hemolysis or liver damage withdrawal of methyldopa is followed as a rule by a rapid recovery

In the decade 1966–75 the Swedish Adverse Drug Reaction Committee which started to obtain reports of adverse reactions in Nov 1965 received more than 300 reports on such reactions with methyldopa Most of these reactions took the form of fever hemolysis or hepatic effects This paper presents some clinical characteristics of these adverse reactions as described in the reports

MATERIAL

In the decade 1966–75 a total of 308 reports were received about adverse reactions in connection with methyldopa treatment where a causal relationship between drug and reaction was considered probable or could not be ruled out This does not include reports involving a combination of drugs

A special study has been performed of the reports of reactions in the form of fever hemolysis or hepatic effects As 13 such reports could not be traced the present survey covers 249 cases

RESULTS

The frequency of the different reactions that were reported after treatment with methyldopa is indicated in Table I Several patients had more than one symptom

Allergic reactions mainly involved the skin (exanthema urticaria) Diarrhea was the most common

Present address The Primary Care Centre St Erik s Hospital S-112 82 Stockholm Sweden

Table I. *Adverse reactions to methyldopa reported to the Swedish Adverse Drug Reaction Committee during 1966–75 (308 patients)*

Fever	166
Hemolysis	67
Hepatic effects	29
Allergic reaction	23
Gastrointestinal symptoms	17
Psychic and neurologic symptoms	13
Other hematologic reactions, incl. pos. Coombs' test	11
Other	16

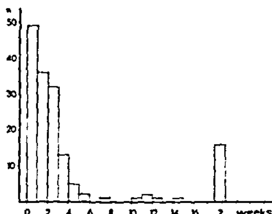


Fig. 1. Duration of treatment before onset of symptoms among patients with fever in connection with methyldopa treatment.

gastrointestinal reaction. Of the 13 reports of adverse reactions from the nervous system, 9 concerned depression. The group "other hematologic effects" includes one case each of thrombocytopenia and leucopenia in addition to four cases of a positive Coombs' test without demonstrable hemolysis.

Fever, hemolysis and hepatic effects

Two-thirds of the patients with fever, hemolysis or hepatic effects in connection with methyldopa treatment were women (Table II). The predominance of women is most marked among the cases of hemolysis and hepatic effects (76 and 74%, respectively). The groups of reactions also differ in terms of median age, this being lowest (57 years) for patients with hepatic effects and highest (69 years) for those who developed hemolysis.

There are also large differences in the length of the interval from the start of treatment to the onset of symptoms. Fever developed as a rule (in 82%) within three weeks (in 117 of 142 cases with a record of treatment dates). There are 13 cases (9%), however, where more than four weeks appear to have elapsed before the onset of fever (Fig. 1). The hemolysis group has the longest period of treatment before symptoms were detected (median 12 months, range two months to several years). The patients with hepatic effects had been treated with methyldopa for periods between one month up to several years (median 6 months) (Fig. 2). The dose at the onset of adverse reactions ranged from 0.125 to 1.5 g/d. The median value for the group with fever is 0.5 g/d, and for each of the other groups 0.75 g/d.

Table II. *Sex and age distribution of patients with adverse reactions with methyldopa (fever, hemolysis or hepatic effects)*

	Fever	Hemolysis	Hepatic effects	Total
Women	98	48	20	166
Men	61	15	7	83
Total	159	63	27	249
Age (y.)				
Median	65	69	57	
Range	34–86	52–86	22–80	

The patients with fever are reported as a rule to have presented a typical clinical picture with a high temperature, which fell back in a couple of days once methyldopa had been withdrawn (8). Several patients underwent provocation tests and developed fever again within 12 hours. In addition to fever, 13 of the reports note pathologic liver tests, in all cases referring to moderately increased transaminase levels—the maximum values for SGOT and SGPT were 204 and 316 IU, respectively (S-ASAT and S-ALAT 3.5 and 5.4 μkat). Eight of the patients had skin manifestations (exanthema, urticaria, erythema nodosum and petechiae) and gastrointestinal complaints accompanied the fever in four. Two patients are reported to have had eosinophilia.

All the patients with hemolysis had a positive Coombs' test. The Hb level rose in all cases once methyldopa had been withdrawn. There are no records of whether and, if so, when the Coombs' test became negative again. The cases of hemolysis after methyldopa that were reported in the period 1965–70 have been analyzed in a previous paper (3).

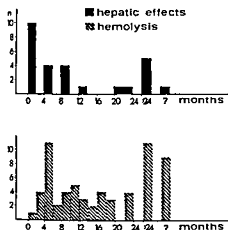


Fig 2 Duration of treatment before onset of symptoms among patients with hemolysis or hepatic effects in connection with methyldopa treatment

The patients with hepatic effects appear to have presented a clinical picture with many similarities to infectious hepatitis in good agreement with the hepatitic reaction that is considered to be typical with methyldopa (4) Icterus was the symptom in a majority of cases which prompted an examination that led to a diagnosis Altogether 180 of these patients (72%) were hospitalized for the symptoms that arose in connection with methyldopa treatment The proportion is highest among those with hepatic effects (85%) and lowest for those with hemolysis (70%) The patients reported in the period 1966–72 have the same proportion (98 of 136 72%) as those reported in 1973–75 (82 of 113 73%)

Methyldopa appears to have been used fairly frequently as the drug of first choice in the treatment of hypertension Almost half of the patients with fever hemolysis or hepatic effects were taking methyldopa as *the only medicine for hypertension* the proportion was higher in the period 1966–72 (54%) than in the later years (39%) (Table III) A comparison between the periods 1966–72 and 1973–75 reveals only moderate differences in the choice of other antihypertensive drugs Beta blockers are introduced furosemide is used to an increasing extent and the number of patients treated with chlorthalidone decreases

DISCUSSION

The nature of several adverse reactions to methyldopa is such that they are often not even brought to the attention of the doctor in charge quite apart from leading to a report Many patients are disinclined to discuss sexual problems for instance with their doctor In other cases the patient may attribute the symptoms particularly those of a psychic nature to other causes than medication Active questioning in this respect yields quite different results from a spontaneous account by the patient (1)

The regulations for reporting adverse reactions in Sweden emphasize the importance of drawing attention to severe and unexpected reactions (9) Such a system leads to a very low frequency of reports on reactions that are common and familiar (e g gastrointestinal complaints after methyldopa) It is thus understandable that the relative incidence of the various adverse reactions which are reported differs greatly from the findings in an intensive study that involves active questioning of the patients

On the other hand the reports usually contain information that may yield valuable clinical knowledge about the reactions The spectra of symptoms of the three adverse reactions to methyldopa that were reported most frequently—fever hemolysis and hepatic effects—are as a rule in good agreement with descriptions in the literature The moderate rise of transaminase that has been reported in some of the fever cases may have to do with the slight direct hepatotoxic effect which methyldopa is assumed to have (6) It is more difficult to explain the differences betwen the diagnostic groups in terms of age and sex One would admittedly expect women to predominate as they appear to be involved more frequently than men in adverse drug reactions in general (2 7) Their particularly marked dominance in the group with hemolysis which is considered to be of an autoimmune nature is likewise

Table III *Other antihypertensive treatment of patients with adverse reactions to methyldopa (fever hemolysis or hepatic effects)*

No of other antihypertensive drugs	1966–72		1973–75	
	n	%	*n*	%
0	75	54	44	39
1	56	41	54	48
≥2	4		8	
?	1		7	
	136		113	

in line with previous experience But the very large female majority in the group with hepatic effects as well as the low median age in this group differ from the usual pattern There are however too few patients with hepatic effects to warrant any definite conclusions An onset of fever not more than three weeks after the start of treatment as reported in most cases also agrees with earlier descriptions But as many as 9% of the present patients first presented this symptom after having been on methyldopa for more than one month which in many cases caused diagnostic difficulties

It is reported in the literature that the onset of hemolysis hardly occurs before methyldopa has been given for at least 6 months (8) In almost one third of the present cases (16 of the 54 with recorded dates of treatment) methyldopa had been given for *not more than* six months It is reasonable however that data on the duration of treatment should differ between series It is seldom possible to establish the actual onset of hemolysis—the record refers to its discovery Large differences can be explained by for instance routines for the frequency with which certain laboratory tests are checked In several of the present cases the hemolysis was diagnosed before the patient had developed symptoms of anemia

The doses of methyldopa that were used in the treatment of the patients who developed fever hemolysis or hepatic effects do not appear to differ from the probable average for this drug The median doses for the various groups are in fact smaller than the defined daily dose which is 1 g 1 methyldopa (5)

The burden which adverse reactions to methyldopa imposes on the health service is illustrated by the circumstance that almost 3/4 of the patients had to be hospitalized for these reactions The reasons for admission to hospital are not always given in the reports but they presumably included a need for nursing (high fever) as well as a need for examinations (indefinite icterus hemolysis)

Methyldopa has been used to a large extent as a drug of first choice in the treatment of high BP The finding that the proportion of patients with methyldopa as the only antihypertensive drug fell from just over half in 1976–72 to about one third in the last three years of the decade seems to be well in line with development in this area But even towards the end of the period it was still common to administer methyldopa as the sole drug against hypertension Of the 13 patients who were reported to have developed fever after methyldopa in 1975 (duration of treatment <4 weeks) six were taking methyldopa as the only antihypertensive drug

Methyldopa has been in clinical use for many years and it is therefore reasonable that a large number of adverse reactions should have been described There is probably also a high incidence of reactions of a type which many patients are disinclined to discuss with their doctor The reactions described here besides adding to the burden on the health service have involved a severe strain on the patients concerned These circumstances should serve as an argument for using methyldopa still less than at present as a routine drug of first choice in the treatment of benign hypertension

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Electrophysiological Methods in Assessing Cardiac Effects of the Tricyclic Antidepressant Imipramine

Leif Brorson and Bertil Wennerblom

From the Division of Cardiology Department of Medicine I Sahlgren s Hospital Goteborg Sweden

ABSTRACT The tricyclic antidepressant agent imipramine was tested intravenously in 8 healthy individuals with respect to its effect on sinus recovery time, intervals in His bundle electrograms and the duration of the repolarization in the right atrium and ventricle judged from the refractoriness and monophasic action potential duration Sinus recovery time was unchanged after the drug The interval between the His bundle deflection and the start of the QRS complex in the standard lead showed no consistent changes but increased to pathological values in two individuals The duration of the repolarization decreased in the atrium—an effect which could be arrhythmia provoking or arrhythmia protecting The duration increased at the ventricular level This effect could explain the antiarrhythmic effect on ventricular ectopics observed in other investigations

In cases of acute poisoning with tricyclic antidepressant agents (TCA) ECG abnormalities have been observed in about 90% of the patients (13) The changes include fatal asystole and ventricular fibrillation supraventricular ectopics and tachycardias ventricular ectopics and conduction disturbances Similar arrhythmias have been observed in some patients on therapeutic doses of TCA (5)

In investigations concerning AV conduction using His bundle electrograms an impaired distal AV conduction was found after toxic doses of TCA (5 15) A similar tendency was observed in some of the patients on therapeutic doses

There are also numerous reports (5) of ST T changes and QT prolongation even in patients on therapeutic doses indicating an impaired repolarization and QT prolongation and ventricular fibrillation have shown connections (14) These observations led to the present study using modern methods for electrophysiological evaluation of the repolarization of the heart muscle Thus the refractory state and the monophasic action potential of the right atrium and ventricle were evaluated before and after a single therapeutic i v dose of the TCA imipramine (Imipramin Hassle Ciba-Geigy Sweden) in healthy males In addition the sinus recovery time was determined and a His bundle electrogram recorded before and after the drug

STUDY POPULATION

Eight healthy male volunteers aged 20–40 years participated The subjects informed consent was obtained None of them had any signs of heart disease as judged from history physical status ECG or chest X ray All had normal serum electrolytes They were not receiving any drug treatment

Sinus recovery time (SRT) was determined after 2 min of atrial pacing with a pacemaker electrode positioned close to the sinus node in most cases A pacing frequency of 120 beats/min was used The SRT was defined as the distance from the last paced P wave to the first spontaneous P wave Corrected SRT (CSRT) was defined as the difference between SRT and the atrial cycle length preceding the onset of pacing (9)

His bundle electrograms (HBE) were recorded as described by Scherlag et al (12) using a bipolar electrode catheter positioned across the tricuspid valve HBE and ECG leads 1 and 2 were displayed on an oscilloscope and recorded on a battery powered 3-channel Mingograph (EM 34 Siemens Elema Sweden) at a paper speed of 100 mm/sec The recordings were analyzed with respect to P–H i e the interval between the start of the P wave in the standard ECG and His deflection in the HBE H–V i e the interval between the His bundle deflection and the start of the QRS complex in the standard lead and H–S i e the interval between the His deflection and the end of the QRS complex in the HBE lead Each value is the mean of the intervals of 5 consecutive beats

Refractory periods were determined using the extra stimulus method (11) The effective refractory periods of

Table I *Changes in cycle length (CL) corrected sinus recovery time (CSRT) and His bundle electrogram (HBE) after imipramine infusion*

P–H = interval between start of P wave in standard ECG and His deflection in HBE H–V = interval between His bundle deflection and start of QRS complex in standard lead H–S = interval between His deflection and end of QRS complex in HBE lead

Subj no	CSRT	HBE P–H	HBE H–V	HBE H–S	CL
1	−5	+8	0	+2	−25
2	−215	−15	+20	+10	−5
3	+80	–	–	–	±0
4	−240	+3	−3	−20	−70
5	−80	+5	−10	−8	+70
6	−160	−20	+10	+47	−305
7	+30	+3	+7	+20	−80

the right atrium (AERP) were determined by high atrial pacing The same pacing position was used for determining the effective AV refractory periods (AVERP) The ventricular effective refractory period (VERP) was determined by apical right ventricular pacing A specially constructed pacemaker (Siemens Elema Sweden) was used for pacing and delivery of premature stimuli The basic cycle length was 600 msec and the stimulus strength ice the threshold value The refractory periods were ermined with a precision of 2 msec The atrial pacing in nection with the determination of AERP and AVERP as performed with the same bipolar pacemaker electrode catheter as was used in the determination of SRT The ventricular pacing was performed with the HBE pacemaker catheter repositioned to the apical region of the right ventricle

Monophasic action potential (MAP) recordings were performed with the suction electrode technique (2 10) with recording in the right atrium (RAMAP) and in the septal part of the right ventricle (RVMAP)

Drug infusion (imipramine 0 5 mg/kg b wt) was performed during 30 min Determination of SRT HBE and the repolarization was carried out before and 10 min after completion of the infusion Care was taken not to change the position of the catheter for atrial and ventricular pacing and to achieve the same position of the MAP catheter before and after drug infusion

RESULTS

Heart rate corrected sinus recovery time and His bundle electrogram

The dose used caused a slight increase in heart rate with a mean decrease in cycle length of about 40 msec

CSRT was for technical reasons determined only in 7 of the 8 individuals It showed a shortening of 5–240 msec in 5 of them and a small increase in 2 (Table I) The change was not statistically significant

HBE was possible to record and calculate in 6 of the 8 individuals The P–H was not consistently changed after the drug (Table I) The H–V increased in 3 decreased in 2 and was unchanged in 1 of the 6 investigations performed A similar increase was elucidated both during spontaneous sinus rhythm and during atrial pacing with a pacing frequency of 100 msec (Table I) The H–V reached pathological values (>55 msec) in 2 individuals after drug infusion The H–S was prolonged in 4 and shortened in 2 subjects

Refractory periods and monophasic action potentials

AVERP could not be determined after drug infusion in two cases in one because of the appearance of a second degree AV block at a pacing frequency of 100/min and in the other because of difficulties in getting proper atrial pacing In the remaining

Table II *Effects of imipramine on the refractoriness of the atrium (AERP) ventricle (VERP) and AV conduction system (AVERP) and on the monophasic action potential of the atrium (RAMAP) and ventricle (RVMAP)*

	AERP	RAMAP	VERP	RVMAP	AVERP
Control					
Mean	242	258	231	270	371
S E M	±11 3	±7 1	±6 2	±5 3	±37 8
After drug					
Mean	216	232	234	282	322
S E M	±6 3	±7 2	±3 9	±5 6	±48 4
Mean difference	−26*	−26*	+3 n s	+12*	−49
n	7	6	8	7	6

* $p<0$ 05 n s = not significant

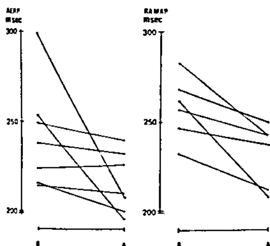


Fig 1 Individual values of the atrial effective refractory periods (*AERP*) and monophasic action potentials (*RAMAP*) before (*B*) and after (*A*) imipramine infusion

six investigations AVERP decreased in five and showed a small increase in one case The mean change was −49 msec (Table II)

AERP decreased (mean −26 msec) in 6 of the 7 investigations performed and was unchanged in one (Table II and Fig 1) RAMAP was determined in six of the seven individuals it could not be recorded in the seventh because of catheter failure RAMAP decreased in all six subjects and the magnitude of the decrease was comparable to the change in AERP (Table II and Fig 1)

VERP was determined in all 8 individuals It was increased in six unchanged in one and slightly de creased in one The drug did not cause statistically significant differences (Table II Fig 2) RVMAP was determined in all but one of the individuals It increased in six and decreased in one The mean increase was 12 msec and statistically significant (Table II and Fig 2)

There were no consistent changes in pacing threshold after drug infusion

No serious side-effects were seen during the investigations When determining the refractory periods occasional ectopic beats were seen and in one case atrial fibrillation was provoked and had to be DC-converted No lasting ventricular arrhyth mias occurred

DISCUSSION

TCA have several actions on the heart at low con centrations the anticholinergic action prevails and at increased levels an adrenolytic action may be seen (5) In addition TCA have a quinidine like effect (3) It is not possible to distinguish between these effects on the basis of changes in ordinary ECG recordings Several reports have appeared on the cardiotoxicity of TCA resulting in various arrhythmias and some cases even proving fatal On the other hand results from animal experiments (3) and in patients (1) have indicated an antiarrhythmic quinidine like effect of TCA There is therefore a need to evaluate TCA and its electrophysiological actions more thoroughly CSRT decreased in most cases This was presumably due to the anticholin ergic properties of the drug (8)

Previous investigations (15) have shown an im paired distal AV conduction and in some cases AV block even after therapeutic doses of TCA Our results vary with respect to impulse conduction within the His Purkinje system but in two healthy individuals the H–V increased to pathological val ues (>55 msec) This effect could be explained by the quinidine like effect of TCA appearing even after these doses A H–V prolongation might also induce the re-entry arrhythmia as seen during treatment with TCA (4)

ECG changes after TCA have included ST changes as well as QT prolongation indicating an impaired repolarization (5) No further evaluation using modern electrophysiological techniques has been performed in man so far

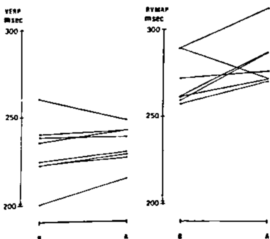


Fig 2 Individual responses to imipramine infusion with respect to ventricular effective refractory periods (*VERP*) and monophasic action potential (*RVMAP*)

The present study revealed a statistically significant decrease in the duration of the repolarization in the atrium judged from AERP and RAMAP after TCA This cannot be explained on the basis of any of the known effects of TCA An anticholinergic or quinidine like effect should have resulted in a prolongation of the repolarization in the atrium (7) The postulated sympatholytical activity of TCA should not have influenced these two variables at all (7) An increase in the repolarization rate might be arrhythmia provoking (4) and thus explain the supraventricular arrhythmias sometimes seen during TCA treatment The underlying mechanism cannot however be explained by the actions of TCA known so far On the other hand a reduction in AERP might also decrease supraventricular arrhythmias (4)

At the ventricular level there was a prolongation of the repolarization with a non significant increase in VERP and a more pronounced increase in RVMAP These changes could be explained by the quinidine like effect of TCA and this action might also explain the antiarrhythmic effect on ventricular ectopics observed in animal experiments (3 6) and in man (1) A prolonged repolarization might however increase the risk of ventricular arrhythmias as in the long QT syndrome (14)

The results of the electrophysiological investigation of the drug are thus difficult to interpret partly because imipramine has different modes of action on the electrophysiological variables studied This varying influence is probably partly dose-dependent (5) and what might be expected at the dose we used is not known in detail even from animal experiments

From the present study however the following hypotheses are put forward 1) The supraventricular tachyarrhythmias observed even after low doses of TCA may be explained by its tendency to shorten the atrial repolarization This effect has not been described earlier 2) TCA might exert their antiarrhythmic action at the ventricular level in the doses used by prolonging the repolarization Furthermore TCA must be used with care in patients with AV conduction disturbances

ACKNOWLEDGEMENT

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The Cardiac Response to a Small i.v. Dose of Dihydralazine, a Safe Drug for Diagnostic Tests?

D. K. Falch and N. Norman

From the Hormone and Isotope Laboratory, Aker Hospital, Oslo, Norway

ABSTRACT. Dihydralazine, given in small i.v. doses, has been of great value in diagnostic tests for unilateral renovascular hypertension where it enhances renin release on the affected side. The acute hemodynamic effects of an i.v. dose of 0.1 mg/kg b.wt. were studied in 14 patients with essential hypertension using a quantitative renographic technique for determination of effective renal plasma flow and radiocardiographic technique for determination of the parameters in systemic circulation. Cardiac index increased from 4.040 to 6.423 l/min m² ($p<0.01$), stroke index from 59 to 66 ml/beat m² ($p<0.05$), heart rate from 70.4 to 96.6 beats/min ($p<0.01$) and left ventricular work index from 1.04 to 1.49 W/m² ($p<0.01$), while mean arterial BP decreased from 125 to 110 mmHg ($p<0.01$) and total peripheral resistance index from 2927 to 1534 10³ N s m⁻³ ($p<0.01$). Effective renal plasma flow and pulmonary plasma volume were unchanged. Peripheral renin activity increased from 0.5 to 1.6 nmol A_I/l h ($p<0.02$). It is concluded that even a small test dose of 0.1 mg/kg of dihydralazine elicits a considerable additional work load on the heart, a circumstance that must be taken into consideration in studies of patients with coronary heart disease.

Hydralazine has a direct action on resistance vessels where vasodilatation (1) and thereby reduction in BP is effected. In addition increased cardiac output and renal blood flow (2, 5, 7, 15, 16) have been observed when hydralazine was given in i.v. doses of 0.15–0.40 mg/kg b.wt. Side-effects (headache, nausea, palpitations, anginal attacks) (9) are frequently seen following these doses.

Hydralazine or the derivative dihydralazine for i.v. administration has been extensively used in diagnostic tests for unilateral renovascular disease with hypertension (9, 10, 12, 14) where a stimulation of renin release on the affected side is obtained. At our laboratory dihydralazine in a dose of 0.1 mg/kg b.wt. has been proven to be suitable for this purpose (12). This small dose of dihydralazine also elicits a lowering of BP and an increase in pulse rate.

As the patients subjected to the test are middle aged, with all degrees of hypertension and have the associated incidence of cardiovascular lesions, a closer study of the additional cardiac load was considered to be of interest.

PATIENTS AND METHODS

Fourteen patients, 13 males and one female, 19–55 years of age (mean 42.5), were included in the study. They were classified as having essential hypertension (stages I or II according to the WHO classification) after clinical examination, urine analyses, ECG, renography, chest X-ray, determination of peripheral renin activity and in some cases also urography and renal angiography. None of the patients took antihypertensive drugs.

The patients were informed in detail of the investigation procedure, as well as possible side-effects of dihydralazine. They volunteered to participate in the cardiac study, which came in addition to the diagnostic renographic examination of their kidneys. They came to the laboratory on two occasions, first for renography and 2–6 days later for radiocardiography. At 8.30 a.m., after a light breakfast and no previous exercise, they rested for 60 min in the supine position, whereafter the studies were undertaken.

Renography

The individual kidney ¹³¹I orthoiodohippuran (¹³¹I OIH) clearance, effective renal plasma flow (ERPF), was determined with a renographic technique as described previously (11, 12). The measurements were performed immediately before and 30 min after an i.v. injection of dihydralazine in a dose of 0.1 mg/kg b.wt.

Radiocardiography

The radiocardiographic study was performed with a technique employing gamma camera, a computer for rapid

Table I *Hemodynamic parameters immediately before (C) and 30 min after (DH) dihydralazine in an i v dose of 0 1 mg/kg b wt*

		Mean	S E M	p
Cardiac index (l/min m²)	C	4 040	0 280	<0 01
	DH	6 423	0.300	
Stroke index (ml/beat m²)	C	59	4	<0 05
	DH	66	2	
Heart rate (beats/min)	C	70 4	3 4	<0 01
	DH	96 6	3 3	
Mean BP (mmHg)	C	125	3	<0 01
	DH	110	2	
Left ventricular work index (W/m²)	C	1 04	0 06	<0 01
	DH	1 49	0 09	
Total peripheral resistance index (10⁵ N s m⁻³)	C	2 927	174	<0 01
	DH	1 534	68	
Effective renal plasma flow (ml/min m²)	C	294	26	>0 05
	DH	339	21	
Peripheral renin activity (nmol A,/l h)	C	0 5	0 2	<0 02
	DH	1 6	0 5	
Pulmonary plasma volume (ml/m²)	C	190	16	>0 05
	DH	191	14	
Interventricular circulation time (s)	C	7 3	0 3	<0 01
	DH	4 5	0 2	

tial uptake and data handling and ^{113m}indium min as the intravascular label as described previ (3) Blood and plasma samples were drawn 10 20 and 30 min after the bolus injection The specific activity of the tracer in these samples allowed for calculation of tracer concentration at the time of injection Radiocardiographies were performed immediately before and 30 min after an i v injection of dihydralazine in a dose of 0 1 mg/kg b wt

The arterial BP was measured on the arm with a mercury sphygmomanometer The same person performed all the BP readings The peripheral renin activity (PRA) was determined by a radioimmunoassay technique for angiotensin I (13)

Calculation

The basis for ERPF calculation was the kidney uptake of ^{131}I OIH 1½-2½ min after bolus injection At this time the renal extraction of ^{131}I OIH is 82 % (4) For comparison of ERPF and cardiac output a corrected flow value of ERPF/0 82 was introduced ERPF referred to in the present study is the sum of that obtained from each kidney and calculated as ml/min m²

The calculation of the radiocardiographic parameters has been described previously (3) The blood and plasma volumes were calculated from the dose of ^{113m}In given the specific activity in blood and plasma being corrected to bolus injection time Cardiac index of plasma (CI_{pl} ml/min m²) was calculated from cardiac index of blood as $CI_{pl}=CI\ C_b/C_{pl}$ where C_b is the radioactivity of ^{113m}In CPM/ml of the whole blood and C_{pl} the corresponding radioactivity in CPM/ml of plasma at bolus injection time The interventricular circulation time for plasma (IVCT) was determined as the time between the peaks of the dilution curves generated for the right and the left ventricles

Mean pulmonary circulation time (PCT) was calculated with the left peak method (3 6) Pulmonary plasma volume (PPV ml/m²) was calculated by multiplying the CI_{pl}/s with PCT (s) (3) Mean arterial BP (MAP) was calculated from systolic (SAP) and diastolic (DAP) pressure as MAP=SAP+2 DAP/3 (mmHg) Left ventricular work index (LVWI) and total peripheral resistance index (TPRI) were calculated as follows

LVWI=[CI (l/min m²) MAP (mmHg) 13 6 g/cm³ 1/1 000] 0 16 (W/m²)

TPRI=MAP (mmHg) 80/CI (l/min m²) (10⁵ N s m⁻³= dyn s cm⁻⁵ m²)

where 13 6 is the specific gravity of Hg 0 16 a converting factor from kpm/min into watt (W) and 80 a converting factor from mmHg/l/min into dyn s cm⁻⁵ Statistical calculations were performed with a Compucorp 445 calculator using Student s *t* test for paired observations

RESULTS

Table I lists the changes in the hemodynamic parameters and PRA elicited by dihydralazine As can be seen cardiac index increased 58 9 % stroke index 11 9 % heart rate 37 2 % and left ventricular work 43 3 % MAP decreased 11 7 % SAP was almost unchanged (from 155 to 153 (S E M ±3) mmHg) while DAP was reduced from 110 to 89 (S E M ±3) mmHg Total peripheral resistance decreased 46 8 % ERPF was unchanged while PRA increased 220 % IVCT was reduced 38 4 % while PPV was unchanged

Nearly all the patients observed transient palpitations and flushing and some of them also experienced nasal congestion and headache The discomfort was moderate and well tolerated

DISCUSSION

According to the present concepts (1 9) the sequence of events following direct action of hydralazine and dihydralazine on the resistance vessels is vasodilatation reduced BP increased sympathetic discharge increased heart rate and cardiac output It is also well established (10 12 14) that PRA increases on i v administration of this drug This effect is demonstrated in our patients with essential hypertension

The small test dose of dihydralazine elicited con

siderable hemodynamic effects on the systemic circulat on In spite of a reduct on in BP and periph eral res stance the calculated left ventricular work increased about 43% due to the large increase in card ac output Ang nal attacks after adm nistrat on of the drug have been observed in patients with coronary heart d sease (7) The increase in left ven tricular work may well have been the cause of these atta ks and our results ind cate that even the injec tion of d hydralazine in a small dose of 0 1 mg/kg b wt m ght be of consequence in pat ents w th ishaem c heart disease

The pulmonary plasma volume was unchanged e en when card ac output and thereby the amount of blood passing through the pulmonary vessels per min ncreased about 59%

Increased renal plasma flow after i v admin stra tion of hydralaz ne has been reported but at h gher doses In the present study the change in ERPF was not statist cally signif cant in spite of the marked increase in card ac output and decrease n BP Renal hemodynamic autoregulat on thus seemed to be intact in these pat ents and with this dose of the drug

It is concluded that the increase in card ac work calls for restrict on in the use of dihydralaz ne in pa ents with ishaemic heart d sease From this po nt of view it is not an ideal drug to use in a diagnost c test

ACKNOWLEDGEMENTS

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Transitory Renal Failure Following Rapid Administration of a Relatively Large Amount of Hematin in a Patient with Acute Intermittent Porphyria in Clinical Remission

G Jeelani Dhar [1] I Bossenmaier R Cardinal
Z J Petryka and C J Watson

From the University of Minnesota Unit for Teaching and Research in Internal Med n Northwestern Hospital Minneapolis Minnesota USA

ABSTRACT Transitory renal failure occurred in a patient with acute intermittent porphyria in clinical remission following i v administration of 1 000 mg hematin The clinical and biochemical picture suggested acute tubular necrosis , which was followed by a prompt and complete return of renal function without any late sequelae The renal failure is thought to have resulted from the presence of circulating free hematin formed as a result of rapid administration of such a relatively large amount Such a complication has not occurred in patients given hematin for acute porphyric relapse in whom much smaller amounts have been infused

Recent studies from our laboratory have provided evidence that hematin administered i v plays a decisive role in the treatment of patients with acute porphyric relapse (8 23 32) Hematin was generally well tolerated and the only side-effects encountered were minor self limited episodes of febrile reactions and phlebitis thought to be related to the type of the hematin solution used and its mode of administration (8) Measures employed to avoid these reactions have been discussed in detail (8) In these studies the amounts of hematin administered have been chosen rather emp as data are unavailable regarding the optimal amount of hematin required to effectively repress the hepatic δ aminolevulinic acid synthetase (ALA S)

In the course of a study designed to provide this information transitory renal failure occurred in a case of acute intermittent porphyria (AIP) in clinical remission following administration of a relatively large amount of hematin as compared with that in other cases We discuss here our observations in this patient and review the pertinent literature on the mechanism of hematin clearance and its possible effects on the kidneys

CASE REPORT

A 40 year-old white construction worker was first found to have AIP at age 19 during a family screening following the death of his 21 year-old sister due to AIP the acute attack having been precipitated by poliomyelitis His 67 year-old uncle has been followed by us for many years and has remained essentially asymptomatic even though he regularly excretes 150–750 mg porphobilinogen (PBG) and 60–70 mg δ aminolevulinic acid (ALA) in the 24 hour urine

The patient had had acute attacks in the past the first in 1963 following administration of sleeping pills while he was hospitalized at another institution for an unrelated illness A second attack occurred about one month prior to the present hospitalization probably induced by intentional drastic reduction in caloric intake with a resultant weight loss of 18 pounds in two weeks In this attack which lasted about 10 days he had severe abdominal pain nausea vomiting and pain in the legs Besides these he

Requests for reprints to C J Watson M D University of Minnesota Unit for Teaching and Research in Internal Medicine Northwestern Hospital 810 E 27th Street Minneapolis Minnesota 55407 USA

[1] *Present address* Department of Medicine Section of Gastroenterology The University of Chicago and The Pritzker School of Medicine Chicago Illinois USA

has had several mild attacks of this general type in the past but more recently increasing in frequency He also had occasional attacks of gout for the past six years He customarily drinks half a case of beer a month and 3–4 times a year he indulges in heavier drinking

The patient like his uncle excretes large amounts of PBG and ALA in the urine whenever studied His serum PBG and ALA ranged between 78–105 and 21–45 μg/100 ml respectively This implies that hepatic ALA S the limiting enzyme of porphyrin biosynthesis is induced at a corresponding level at all times Even though asymptomatic the patient was very much interested to know about the effects of hematin on his disease and in particular whether hematin administration would predictably terminate an acute attack in the event that he had one as had been noted in others (8 23 32) As part of our continuing effort to learn more about the effects of hematin administration and its clearance from the circulating blood the patient volunteered to receive hematin according to the protocol outlined below After obtaining an informed consent a study was designed to ascertain 1) the effects of hematin on chemical porphyria in the absence of symptoms 2) the relation of the initial concentration of hematin to the degree of repression of ALA S and 3) the mechanism of clearance of hematin from circulating blood our previous studies (24) having suggested a clearance curve with an initial fast and a later slow component

METHODS

Following the collection of baseline data for PBG and in blood and urine and for hematin and hemopexin in m the patient (b wt 82 kg) was hospitalized on April 1974 for hematin administration Four hundred mg of ematin in 50 ml of solution pH 8 0 (8) was injected i v over 5 min Blood samples for determinations of hemopexin and hematin were drawn before 5 min after the completion of injection and at frequent intervals during the 48-hour hospitalization then daily for six days on an outpatient basis Also PBG and ALA in serum and urine were determined daily

On April 18 he was rehospitalized and given 1 000 mg of hematin in 135 ml of solution (8) i v over 15 min Samples of blood and urine for determinations of hematin hemopexin PBG and ALA were collected at appropriate intervals PBG and ALA in urine were determined by the method of Mauzerall and Granick (17) and in serum by the method of Miyagi et al (18) Concentration of hematin in and its clearance from the circulating blood was measured as described previously (8) Hemopexin determinations were performed by Dr U Muller Eberhard Scripps Clinic La Jolla California using the radial immunodiffusion method of Mancini The rest of the pertinent determinations in blood and urine were performed in the regular clinical laboratory using automated systems in most of the instances

RESULTS

During the beginning five day control period the mean concentrations of PBG and ALA in serum were 65 and 28 μg/100 ml and in urine 86 and 22 mg/24 hours respectively Following the administration of 400 mg hematin the PBG and ALA in serum declined promptly to a level of 36 and 4 μg/100 ml respectively It was of interest to note that although the serum PBG returned to prehematin level by 72 hours the serum ALA remained at a lower concentration throughout most of this study period (Fig 1) Similar reductions were noted in urinary PBG and ALA (Fig 1) These effects corresponded closely to the time hematin was present in the circulating blood There were no untoward effects

The initial phase of the administration of 1 000 mg hematin in 135 ml (April 18 at 8 00 a m) was uneventful However when almost 100 ml had run in the patient complained of dizziness and tingling and pain in fingers and toes Hyperventilation was not observed The pulse became slow but regular recorded briefly at 48/min after which it returned to 60 The BP monitored frequently was steady at 110/76 mmHg By the time the injection was completed there was severe colicky abdominal pain and sweating The dizziness and tingling and pain in fingers and toes disappeared in the next 15 min however the abdominal pain was only partially relieved when he induced vomiting Thirty minutes after the infusion was completed there was a transient rigor and this was followed by a rise in temperature to 101°F There was no respiratory distress tachycardia or fall in BP at any time He went to sleep following this and on waking at 12 00 he was feeling better without any of the above mentioned symptoms The temperature returned to normal by the next morning and remained so except for a brief rise to 100 6°F that evening

Although the patient remained asymptomatic from then on it was noted that he had developed oliguria The urinary output in the 24 hours following hematin administration was only 200 ml Associated with this there were chemical findings of uremia the development and course of which are shown in Fig 2 The urine was initially almost black but cleared rapidly over the ensuing 36 hours (see below for details) On 4/19/74 at 2 00 p m 1000 ml D_5W with 50 mEq $NaHCO_3$ were infused over one hour to exclude prerenal renal failure and attempt to alkalinize the urine By 6 00 p m he had a urinary output of only 30 ml At this time 40 mg ethacrynic acid and 50 g mannitol were given i v without much result At 8 00 p m 160 mg

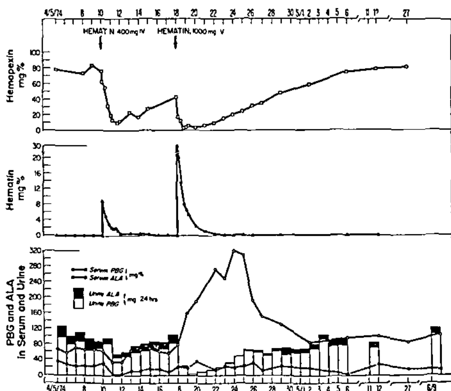


Fig 1 Effect of i v hematin on serum hemopexin and serum and urinary ALA and PBG in a patient with acute intermittent porphyria in clinical remission

ethacrynic acid was given which resulted in 130 ml of urine and at 9 00 p m the dose of ethacrynic acid was increased to 320 mg During the following 12 hours he had a urinary output of 432 ml On 4/20/74 at 2 00 p m he was again given 160 mg ethacrynic acid followed by 320 mg at 3 0 From then on there was increasing urinary with gradual return of the blood urea and creati to normal values (Fig 2)

A plain X ray of abdomen taken on 4/24/74 re

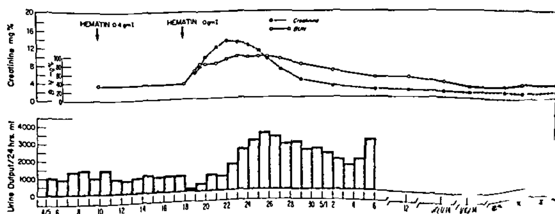


Fig 2 Levels of serum creatinine blood urea nitrogen (*BUN*) and urinary output before and after hematin administration

vealed bilaterally enlarged kidneys Throughout this period the patient remained completely asymptomatic and felt well The initial dark urine passed during the two days following hematin administration was noted to contain 13 8 g/l of protein 1–25 RBC/HPF and 2–5 WBC/HPF and granular casts Hematin was readily demonstrable spectroscopically in this urine identified by addition of $Na_2S_2O_4$ (λ max 557 nm) On electrophoresis it was noted to be associated with albumin

There was a striking paradoxical increase in serum PBG following the hematin administration It gradually increased from its prehematin level of 80 μg/100 ml until it peaked at 320 μg/100 ml (Fig 1) However the same was not true of the serum ALA which did not change significantly The reason for these differences is discussed later The serum hematin concentration following the administration of 1 000 mg peaked at 30 mg/100 ml It decreased rapidly reaching 6 7 mg/100 ml at 24 hours and 2 4 mg/100 ml at 48 hours (Fig 1)

Plasma hemopexin was rapidly depleted following hematin administration (Fig 1) It decreased from a level of 76 to 10 5 mg/100 ml in 41 hours after the initial 400 mg of hematin whereas the fall was more rapid after 1 000 mg hematin i e from 3 2 mg/100 ml in 18 hours (Fig 1) There was a l increase in the hemopexin levels after the l decline the rate of increase being similar in both instances (132 hours for 50% restoration of prehematin levels) The reciprocal relationship between the levels of circulating hemopexin and hematin is self evident (Fig 1)

DISCUSSION

The reduction in the levels of PBG and ALA in serum and urine following the administration of 400 mg hematin occurred in a predictable manner as anticipated from our previous observations (8 23 32) The paradoxical increase in the serum PBG following 1 000 mg hematin was obviously due to the transient renal failure plus continued conversion of ALA to PBG Goldberg (13) found that following i v administration of PBG in rats it was rapidly and mainly excreted in the urine The rate of clearance was calculated to be 0 82 ml/min a figure closely resembling that of inulin clearance in the rat suggesting that the renal clearance of PBG was mainly via glomerular filtration Goldberg (12) extended these studies to patients with AIP and noted that the rate of renal clearance of endogenous PBG in these patients was identical to that of creatinine and inulin determined simultaneously These observations were subsequently confirmed by Druyan et al (9) However these authors found that in their 4 normal controls the renal clearance of endogenous PBG was only 3–10% of creatinine clearance From this they concluded that under physiological conditions there must be a significant tubular reabsorption of PBG It is however possible that at these relatively low levels most of the circulating PBG is protein bound and thus not being filtered at the glomerulus With increase in plasma PBG concentration as in porphyric patients this low binding would be exceeded and the PBG now mostly in its free state would be filtered and subsequently excreted Thus any reduction in glomerular filtration rate would result in a decreased renal clearance and consequent accumulation of PBG in the blood as occurred in our patient

The renal injury which occurred in this patient after 1 000 mg of hematin was scarcely expected in view of present knowledge as to disposal of hematin from the circulating blood as well as previous experience with hematin infusions (see below) Heme formed either endogenously or administered as hematin is rapidly bound by hemopexin the heme binding β globulin of normal serum and albumin (14 15) As hemopexin has much greater affinity for heme than albumin most of the heme is initially bound by hemopexin The circulating hemopexin however averages only 77 mg/100 ml (14) sufficient to bind only about 18 mg hematin (based on 1 1 molar binding ratio) (15) Consequently above a serum hematin concentration of 0 6 mg/100 ml all the hemopexin would have been depleted and further binding would relate to albumin which binds hematin at least on a 1 2 molar basis (26) As such 125 g of circulating albumin should be sufficient to handle up to 2 6 g hematin In other words at least theoretically hematin up to a serum concentration of about 100 mg/100 ml would be protein bound It does not follow however that one can administer such large quantities of hematin at any one time and expect it all to be protein bound as that would depend upon the time needed for such an equilibration

From the studies of Muller Eberhard et al (19–21) Sears and Huser (29) and Snyder and Schmid (30) in a variety of experimental animals and those of Sears in man (28) it can be concluded that hematin is rapidly cleared from the circulation

by the liver as the heme-hemopexin complex Albumin acts only as a temporary storage site from which hematin is transferred to hemopexin at the rate at which the latter is made available Such an in vivo transfer of heme from methemalbumin to hemopexin has been demonstrated (19–21) Liem et al (16) have shown that while hematin decreased the $t^1/_2$ of hemopexin it had no effect on albumin or heat inactivated hemopexin Thus a rapid depletion of plasma hemopexin following the administration of hematin would be anticipated and was observed in the present case (Fig 1)

That the liver is the main or even the exclusive site of disposal of hematin has been adequately documented by a number of studies Thus using ^{59}Fe or ^{3}H labelled heme Muller Eberhard et al (19 20) noted that in rats and rabbits regardless of the form in which heme was injected radioactivity accumulated only in the hepatocytes whereas none was present in the spleen kidney lung or bone marrow Snyder and Schmid (30) obtained similar results in rats given ^{14}C hematin That the same holds true in the case of humans was shown by Sears (28) and Liem et al (16) who noted that i v administration of ^{59}Fe hematin in man was followed by a rapid accumulation of radioactivity over the liver whereas only slight increases occurred over the spleen and sacrum However evidence has been described pointing to hematin uptake in the erythroblasts in congenital erythropoietic porphyria (31) There was no detectable heme pigment or radioactivity in the urine of these subjects even in those cases where hemopexin had been largely or entirely depleted prior to the hematin administration Thus the clearance of hematin from the circulating blood would be determined by the amount and rate of administration the availability of hemopexin and the hepatic clearance

From the foregoing and from earlier studies (2 22) in which hematin in comparable amounts was given i v in human subjects renal toxicity as seen in the present case was not anticipated Neither has there been any suggestion of similar side-effects in any of the other porphyric patients receiving hematin in this laboratory (8 23 32) the amounts used however have been much smaller and the rate of infusion slower than in the present case Duesberg (10) Fairley (11) and Sears (27) also do not mention toxicity ascribable to hematin in their human studies even in cases with some hepatic dysfunction

Brown s early observations (4–6) on malarial pigment led him to believe that this was hematin and that it was responsible for the febrile reactions (5) the degree being dose related an amount of 10–15 mg/kg causing a rise of 3–3 5°F The greatest elevation of 4 9°F was observed in an animal that received 18 mg/kg It must however be pointed out that even the sodium bicarbonate solution that Brown used as a vehicle for hematin administration produced similar dose related febrile reactions although to a lesser degree Also the hematin solutions were not of uniform character some of the solutions being turbid due to colloidal suspensions of hematin To avoid inclusion of pyrogen we have found it essential to crystallize and recrystallize hemin prior to preparation of the solution for i v administration (8) otherwise pyrogens are often detectable by the standard pyrogen test in rabbits (25) Brown (6) observed that administration of hematin at 20 mg/kg was consistently followed by albuminuria and appearance of granular and hyaline casts in urine Similar changes although to a considerably lesser extent could be detected at a dose of 10 mg/kg

Anderson et al (1) noted essentially similar results in dogs that received hematin i v in amounts producing an initial plasma concentration of 10–72 mg/100 ml They also observed that marked toxic reactions and death occurred in animals given injections too rapidly Marked vascular changes consisted of generalized vasodilatation hemorrhages and thromboses Hemorrhage in the subarachnoid space and central nervous system causing convulsions and subendocardial and myocardial hemorrhages were frequent The kidneys also participated in this generalized vascular reaction

Corcoran and Page in an important but little known study (7) observed a transitory renal hyperemia in dogs given hematin i v at 14 5 mg/kg at a rate of 0 28 mg/kg/min A larger dose of 23 7 mg/kg at 0 49 mg/kg/min caused intense efferent arteriolar vasoconstriction with resultant increase in intraglomerular pressure causing azotemia and proteinuria At a still higher level 32 6 mg/kg at 0 835 mg/kg/min there were generalized petechial hemorrhages with ischemia of glomeruli and tubular degeneration leading to shock and death In the present case 1000 mg of hematin were administered at a rate of 0 8 mg/kg/min It is probable that the transitory abdominal distress was related to vasoconstriction and the hematin-contain-



Fig 4 Positive immunofluorescence staining for IgA in a frozen section from kidney biopsy The figure shows a segment of a glomerulus and IgA seems mainly to be located in granular deposits along the capillary basement membrane (×672)

biopsy from involved skin The reason may be that also clinically the patient s illness was inactive and the immunological activity may have been burned ut The findings of mitochondrial and smooth uscular antibodies strengthen our assumption of an immunological disease in this patient They might however be secondary to previous cellular damage

The impaired renal function demonstrated by our results of clearance of PAH and inulin is consistent with the renal biopsy findings DH may therefore be associated with glomerular damage caused by immunocomplex deposits as are probably the skin manifestations It is thus important to examine patients with DH for proteinuria Renal biopsy in such cases may be of significance for differentiating subgroups of DH as has been suggested (4) and may yield new information on the pathogenesis of glomerulonephritis

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REVIEW ARTICLE

Proteases and Protease Inhibitors in Chronic Obstructive Lung Disease

Sten Eriksson

From the Department of Internal Medicine University of Lund Malmo General Hospital Malmo Sweden

Chronic obstructive airway disease comprises several clinically more or less distinct entities such as asthma chronic bronchitis and emphysema The obstruction arises from bronchial narrowing due to either structural narrowing or increased tone and/or to destruction of lung parenchyma This destructive element must be present to justify the term emphysema Emphysema is characterized not only by a supranormal increase in the size of air spaces distal to the terminal bronchiole but also by destructive changes in the alveolar walls (WHO 1961) These destructive changes may be restricted to the respiratory bronchiole (centroacinar or centrilobular emphysema) as opposed to a more extensive destruction of the whole acinus (panacinar) This destructive element is lacking in diseases related to pure hyperinflation i e status asthmaticus Panlobular emphysema is seen most often in patients with so-called type A disease (emphysematous type) with a steadily increasing exertional dyspnea and sometimes weight loss as dominating symptoms Hypercapnia is either absent or occurs late Type B (bronchitic) chronic airway obstruction is intimately associated with chronic bronchitis has more pronounced disturbances of blood gases and secondary polyglobuly Histologically the emphysema is predominantly of the centrilobular variety although a combination is often found The obstructive component can be quantified objectively by simple measurements

The patient with pure type A disease can exhibit a severe degree of obstructive ventilatory impairment but the mechanism is not primarily due to structural narrowing of the bronchi During expiration the increased intrathoracic pressure will exceed intrabronchial pressure resulting in airway collapse which is not readily overcome by further expiratory effort Elastic recoil is responsible for raising intrabronchial pressure above the intrathoracic during expiration Loss of elastic recoil can be considered as a functional counterpart of parenchymal loss or destruction in emphysema of any kind

Since Laennec s first description of the clinical and pathological anatomy of emphysema some 150 years ago the pathogenesis of the disease has been investigated mainly from a mechanical point of view Neither experimental nor pathoanatomic studies have however produced evidence for the assumption that an increase in pressure in the lung tissue distally to a partially or completely occluded bronchial segment is of importance for the destruction of alveolar septa characteristic of the disease Linskog s demonstration in 1932 of the so called collateral ventilation and Gough s post mortem findings in 1953 in fatal status asthmaticus argue against the assumption that an increase in pressure is of essential pathogenetic significance Neither do the notoriously unsuccessful attempts to induce emphysema mechanically in experimental animals support this hypothesis

α_1-Antitrypsin deficiency and the proteolytic theory of emphysema

Advances in the understanding of cellular and tissue mechanisms have frequently paralleled the recognition of inherited defects Discovery in the 60s that the deficiency of α_1 antitrypsin (α_1 AT) was associated as a rule with the early appearance of pulmonary emphysema provoked the conclusion

Upon editorial request references have been largely limited to reviews and similar papers

Abbreviations α_1 AT=α_1 antitrypsin α_2-M=α_2-macroglobulin STIC=serum trypsin inhibitory capacity

that emphysema was probably the end result of extensive tissue destruction of the lung (2 3 13)

In our first publication on this subject (13) where the biochemical characteristics of α_1 AT deficiency as a new inborn error of metabolism were defined we pointed out its possible association with degenerative lung disease The hereditary nature of the disease and its association with familiar emphysema were first described in a paper published in this journal in 1964 (2) At that time the genetics of the entity seemed very simple homozygotes with approximately 15% of normal plasma level having the mutant gene in double dose and heterozygotes with 60% of normal level having only one abnormal gene These studies were based on estimation of STIC (serum trypsin inhibitory capacity) The discovery of other genetic variants however soon made the picture more complex α_1 AT is genetically polymorphic On electrophoresis at an acid pH each α_1 AT variant produces a distinctive eight banded pattern More than 20 genetic variants can be recognized They have been given letter designations Pi (protease inhibitor) B C D M Z etc according to their electrophoretic mobility (4) The genetic types are inherited in an au codominant fashion each gene being ex The Pi^M allele is the most common and s in a normal amount of plasma α_1 AT Of the ognized Pi alleles Pi^P Pi^S and Pi^Z all result in decreased plasma levels approximately 25 65 and 15% respectively of the level with the normal Pi^M gene (100%) The very rare Pi (Pi null) allele is *seen in individuals with no detectable plasma* α_1 AT Clinically classical α_1 AT deficiency (Pi^{ZZ}) is the most important genetic variant The reported frequencies of the Pi^Z alleles vary considerably in different geographic areas The allele is common (frequency 0 026) in the Scandinavian countries as compared to the USA (0 013) and France (0 006) In Sweden approximately 1 of 1 700 individuals is a Pi^{ZZ} homozygote and 1/20 a Pi^{MZ} heterozygote

Patients with the classical type of severe α_1 AT deficiency (Pi^{ZZ}) exhibited several distinctive features The age at onset was relatively low preceding chronic bronchitis was often absent (primary emphysema) weight loss often prominent and hypercapnia a late complication All were features fitting with the type A disease and autopsy findings using the *Gough* technique also confirmed the presence of a typical panacinar emphysema in these patients Another characteristic feature was the predominant localization in the lung bases but often a well preserved upper lobe function (3) Pronounced loss of elastic recoil could be observed early even in asymptomatic patients Clinical and pathological studies have stressed the fact that chronic bronchitis not infrequently complicates the underlying emphysema and that these patients may not present a picture of pure emphysema According to data from a recent compilation of more than 250 Swedish cases the breakdown between these two modes of clinical presentation seems to be close to 1 1 (Larsson to be published)

In the early 60s not much was known about *protease inhibitors in human plasma Camus and Gley* had already in 1897 reported on the presence of antiproteolytic activity in blood but not until 50 years later was the complexity of the plasma protease inhibitor system revealed by the pioneer work of Grob (1949) and Schulman (1952) Jacobsson localized the major trypsin inhibitor in the electrophoretic α_1 zone in 1955 and Bundy and Mehl isolated the inhibitor in 1959 Schultze et al had isolated a 3 5S glycoprotein in 1955 but did not report its ability to bind protease until early 1962 when the deficiency state was detected To estimate antiproteolytic activity trypsin was used in a two step procedure Plasma was first incubated with a known excess of trypsin and the unbound trypsin was measured in a second step using a suitable substrate This procedure measures the sum of all protease inhibitors in plasma (total STIC) irrespective of the firmness of the bond between added *protease and serum inhibitor*

It was evident to early investigators in the field that under physiological conditions there was little chance that pancreatic trypsin could be significant in the pathogenesis of emphysema But there was evidence from investigations published by Grob in 1949 that other proteases than trypsin could be inhibited by α_1 AT Granulocytes were therefore suggested as one possible protease source This hypothesis was strengthened by the knowledge that lung parenchyma can sequester large amounts of granulocytes Kueppers and Bearn (10) also provided early experimental support for the concept that α_1 AT can inhibit leucoproteases *An alternative protease source suggested was the alveolar* macrophage also known to contain proteases Based on available indirect evidence it was suggested that emphysema in α_1 AT deficiency was the result of uninhibited proteolytic digestion of lung

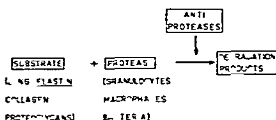


Fig 1 Proteolysis in emphysema

parenchyma by proteases liberated from endogenous cells such as granulocytes and alveolar macrophages. The proteolytic theory could for the first time provide a satisfactory explanation of the destructive element in emphysema and thus gave the pathogenetic thinking in this disease a new direction. During the last 10 years the proteolytic theory has been widely accepted and knowledge in the field has been rapidly expanding. As will be evident most new data fit very well with the theory but there are some unclear points.

This review will summarize some of the present knowledge in the field with respect to clinical findings, physiology and biochemistry. Let us first consider the pertinent reactants involved (Fig 1). The substrate (lung tissue components) is attacked by the enzymes (proteases from various sources) resulting in a degradation of substrate to functionally defect molecules leading to disturbed physiological function and disease. In α_1 AT deficiency this reaction proceeds relatively rapidly to the right due to low levels of one important modulator, namely α_1 AT.

The substrate(s)

For a penetrating review of the biochemistry of lung connective tissue the reader is referred to Hance and Crystal (5). Of the three main components of lung connective tissue—collagen, elastin and proteoglycans—elastin is probably the primary target of attack. Morphologically there is ample evidence of destruction of this component resulting in altered elasticity. However, in biochemical terms it has been difficult to demonstrate a reduced quantity of total elastin in emphysema. Even in α AT deficiency the total amount of elastin seems to be only marginally reduced and therefore much work has been directed towards tentative qualitative biochemical differences between normal and emphysema elastin. So far the results are conflicting. Several possibilities remain open, e.g. the increased ratio of polar/non polar amino acids could be due to a selective destruction on non-polar regions of elastin. There are no convincing data supporting quantitative or qualitative changes in lung collagen in emphysema and the role of proteoglycans is still more unsettled. Elastin probably is the most important component for future study. Before we can fully accept the proteolytic model in emphysema we must be able to prove that elastolysis actually occurs in vivo. At present we have only indirect biochemical evidence of enhanced elastolysis even in α AT emphysema. One possible approach to this dilemma is to study and try to quantify the reaction products in the process illustrated in Fig 1. Reserach in experimental emphysema may help to identify, characterize and measure elastin degradation products by chemical and/or immunological means (19).

The enzymes

Interest has been focused mainly on granulocytes and alveolar macrophages as potential sources of proteolytic enzymes. Turning first to granulocytes much progress has been made in understanding the biochemistry of these cells with special reference to the high content of proteases and their properties, but knowledge of the kinetics of the granulocytes has not expanded to the same degree. Much attention has been paid to the ability of lung tissue to sequester granulocytes. The pulmonary circulation is certainly capable of rapidly removing vast quantities of granulocytes from circulating blood and can serve as a storage organ as shown by Bierman et al, i.e. this process is reversible and does not necessarily mean that a significant amount of the cells is actually destroyed in the lungs. One of the weak points in the proteolytic theory of emphysema is this lack of knowledge of the ultimate fate of granulocytes in the lung. What proportion of granulocytes marginated in the lung capillaries passes through the capillary walls into the interstitial fluid and perhaps further into the alveoli, and what proportion of cells disintegrate or release enzyme in this area? The cytologist examining sputum samples representative of the most distal airways is not impressed with a great num[illegible] ulocytes even in the heavy cigarette sm[illegible] er with the increase of macrophages. [illegible] despite a lack of firm experimental e[illegible] is probably correct to assume that the [illegible] important site of granulocyte destru[illegible] then

S. and

struction so prominent in the lung bases in the deficiency state? No explanation is at hand except for the well known predominance of perfusion per unit lung volume in the basal portions Another item that has not been seriously discussed is the absence in α_1 AT deficiency of pathological changes due to hypothetical protease excess in other organs than the lungs There is equally good evidence that organs supplied by the splanchnic circulation act as granulocyte sequestors One explanation may be that the exchange rate of interstitial fluid is much more rapid in these organs than in the lungs I will not discuss here in detail the peculiar features of the liver disease which occurs in a small proportion of α_1 AT-deficient children and adults (26) The end result of this process is fibrosis or cirrhosis of the liver At present there is no experimental evidence that this complication is due to uninhibited protease excess It seems to be intimately correlated with the Pi^{Z} phenotype and its typical PAS positive inclusion bodies in contrast to emphysema which appears in other phenotypes with low plasma levels (Pi^{--}) but without inclusions in the hepatocytes

In 1968 Lazarus et al (15) and Janoff and Scherer 8) defined the presence of collagenase and elastase vities within the granulae fraction of human ulocytes Lieberman and Gawad (18) initiated portant studies using extracts of leucocyte enriched purulent sputum to demonstrate their ability to digest connective tissue components of hamster and human lung tissue They could also demonstrate that this digestive activity was effectively inhibited by human α_1 AT During recent years much work has been dedicated to isolation and characterization of these proteases The neutral proteases elastase and collagenase and the chymotrypsin like cationic protein have all been isolated from granulocytes and characterized (22) Specific antisera have been produced permitting their quantification in biological fluids and studies on their interaction with antiproteases discussed in more detail below In adult man approximately 50 ml of granulocytes are produced each day Proteolytic enzymes constitute 5% of their cell mass Considering the relatively short half life of granulocytes approximately 1 g of elastase and collagenase is produced each day These enzymes are capable of degrading elastin and collagen in various organs and obviously their unopposed action i e in the absence of inhibitors would be disastrous

The pulmonary alveolar macrophage is an alternative source of protease which has received increasing attention It is rich in cathepsins (lysosomal proteases with an acid pH optimum) but also contains elastolytic and collagenolytic enzymes with a neutral pH optimum Although less well characterized than the granulocytal enzymes it is of considerable interest in the discussion of the proteolytic theory of emphysema pathogenesis There is increasing evidence although no prospective studies are yet available that cigarette smoking in the Pi^{ZZ} deficiency state is of major prognostic significance It was observed in the early clinical studies of these patients that non smokers had a great chance of surviving to a high age without any symptoms of emphysema In smokers the alveolar lavage fluid has a 4–5 fold increase in the number of macrophages Furthermore it has been shown that the macrophages of cigarette smokers have a higher elastase like activity than those of non smokers Taking these data into account Harris et al (6) found 10 times more elastase like activity and 18 times more protease activity within macrophages from cigarette smokers lungs It is equally difficult to disregard these findings when discussing the fate of the patient with severe α_1 AT deficiency as that of the individual with intermediate α_1 AT deficiency Much effort has been devoted to proving that individuals with intermediate deficiency (Pi^{MZ} Pi^{SZ} Pi^{SS}) are at increased risk of developing emphysema Space does not allow a detailed discussion of this matter (for a review see Morse et al (20)) Our personal opinion is that Lieberman s concept is correct when he states that an additive effect of cigarette smoking and intermediate deficiency predispose to a premature emphysema development In a recent investigation of an unbiased population sample of 50-year old men (11) we found no physiological abnormalities whatsoever in the non smoking Pi^{MZ} individual in contrast to clearly decreased recoil pressure and increased residual volumes in smokers The clinical significance of these findings is difficult to evaluate The alveolar macrophage may play a critical role in this premature emphysema development but other hitherto unknown alternative mechanisms should be sought

The antiproteases and their function

To understand the link between emphysema and the action of free proteases we might consider some basic facts about the main protease inhibitors in plasma (for a review of their biochemistry see

Laurell and Jeppsson (14) Together α_1 AT and α_2-macroglobulin (α_2-M) comprise more than 90% of serum inhibitory capacity α_2 M has a very large molecular size (625 000) as compared to α_1 AT (56 000) so that in spite of roughly equal plasma levels (2 0 respectively 1 3 g/l) the molar concentration of α_1 AT is about 10 times that of α_2 M

Although of minimal biological interest in emphysema pathogenesis trypsin was for a long time used as a model enzyme in elucidating the reaction kinetics between protease and antiproteases After the discoveries that α_1 AT could inhibit granulocyte elastase development has been rapid In a long series of papers Ohlsson and Delshammar (21) using monospecific antisera against granulocyte proteases and Laurell s crossed immunoelectrophoretic technique have contributed very convincingly to our present concept of these interactions in health and disease Both α_1-AT and α_2-M have a broad inhibitory spectrum α_1 AT inhibiting serine proteases on a 1 1 and α_2-M on a 1 2 molar basis Granulocyte elastase and collagenase are complexed by α_1 AT but elastase is bound with higher affinity Due to its smaller molecular size α_1 AT occurs mainly extravascularly in the interstitial fluid in contrast to α_2 M which occurs mainly intravascularly lung lymph being an exception It is therefore evident that α_1 AT is the main intercellular protease inhibitor available in the first line of defense against free proteases especially elastase α_2-M has a still wider inhibitory spectrum resulting in irreversible inactivation Barett and Starkey (1) have suggested that the binding is initiated by a proteolytic attack on the molecule resulting in a conformational change with entrapment of the enzyme within the α_2-M molecule A transfer of enzymes from α_1 AT to α_2-M occurs in vivo The conformational change of the molecule results in a rapid elimination of the complex in the RES One can thus imagine α_2-M as a final common pathway in a chain with the purpose of eliminating potentially harmful free proteases Deficiency of α_2-M has never been described and if it exists is probably not compatible with survival

Protease inhibitor complexes are rapidly eliminated and cannot be seen in plasma using standard methods but are easily demonstrated in purulent ascitic fluid and other exudates rich in inflammatory cells Recent studies by Ohlsson and Tegner (23) showed that substantial amounts of proteolytic enzymes are released from granulocytes in purulent sputum Both free enzymatically active and inhibitor bound proteases could be demonstrated It seems quite obvious that an excess of free proteases in purulent sputum could damage lung connective tissue components and ciliary function even in individuals with normal levels of protease inhibitors The delicate balance between protease and inhibitor thus can be disturbed in two principally different ways in the genetic deficiency states the imbalance is due to insufficient production of inhibitors in contrast to excessive release of free proteases in the patient with bronchitis or bronchiectasis

However the exact role of α_1 AT at the alveolar level in man is not clear Bronchial secretion contains—in addition to inhibitors derived from plasma—a low molecular inhibitor produced locally and not present in plasma which forms complexes with granulocyte elastase Interestingly this inhibitor first described by Hochstrasser et al (7) accounts for about 90% of the molar inhibiting capacity of normal bronchial secretion (27) Its physiological role in the local defense system must await further studies As with other protease inhibitors it is not only the molar concentration of the inhibitor that determines its effectiveness as an inhibitor but also its affinity constant

Many patients with α_1 AT deficiency develop emphysema without any evidence of preceding infections and without signs of bronchitic element In these individuals we have to look upon emphysema development as a result of a dysbalance between the amount of proteases released at a normal rate from endogenous cells such as granulocytes and the amount of available extracellular inhibitors In cigarette smokers we can expect a relatively increased release of proteases from both granulocytes and above all alveolar macrophages accumulating in lung parenchyma At present our knowledge of the interaction between various inhibitors and macrophage proteases is incomplete Conflicting reports on the ability of α_1 AT to inhibit macrophage elastase have appeared

Experimental support for the proteolytic theory

Independently of the investigations started in 1962 on the relation between α_1 AT and emphysema Gross et al in 1965 induced emphysema experimentally in the rat by endotracheal instillation of papain a proteolytic enzyme from plants for which

the animals have no natural inhibitors Their investigations meant a breakthrough in experimental emphysema research in that they had found a method for producing emphysema which in several respects pathoanatomically resembled human emphysema The subject has recently been reviewed by Lieberman (17) The papain induced emphysema is characterized by a marked loss of elasticity but little or no bronchial obstruction and as far as the mechanics of the lungs are concerned resembles the early phase of α_1 AT deficiency The exact mechanism behind papain emphysema is unknown The development of emphysema can be very rapid and is preceded by an intense inflammatory reaction Release of proteases from inflammatory cells may thus be directly responsible for emphysema development Furthermore there is recent evidence that papain cleaves off an N terminal peptide from α_1 AT resulting in loss of its inhibitory capacity Of great importance is the observation that once emphysema is established it seems to progress without further exposure to papain This may suggest that other mechanisms than proteolysis are involved

A more physiologic source of proteases is ulocytes and in 1972 Mass et al were able to ...ce emphysema in dogs by aerosolization with ...omogenates of leucocyte preparations There was a correlation between the ability of their homogenate to induce emphysema and the protease content Pancreatic elastase was used for the same purpose by Kaplan and coworkers in 1973 and more recently purified human leucocyte elastase has been shown to initiate emphysema development in animals (25) A variety of other enzymes of bacterial origin are also capable of producing emphysema Although the animal emphysema closely mimics the human disease and strongly supports the proteolytic theory we must be careful when interpreting the results The mode of administration by intratracheal inhalation or aerosolization is unphysiological Furthermore we must consider that the various proteases used have very little in common except proteolytic activity In experiments with pure human leucocyte elastases administered to hamster the most striking effect was extensive lung hemorrhage In the surviving animals emphysema developed later but was less pronounced as was the evidence of elastolysis when compared to the effect of pancreatic elastase (25) The leakage of plasma inhibitors into hemorrhagic lung areas and the different affinity of granulocyte and pancreatic elastase to the low molecular inhibitor may explain the results but it has also been shown that these two elastases produce different degradation products when incubated in vitro with elastin (24) However the overwhelming mass of information available points to elastin as the main target and elastolysis as the common denominator in experimental emphysema and the model is certainly a promising research tool not least in the evaluation of drugs with potential antielastolytic effects The model has also provided valuable information on the relationship between the mechanical properties of the lung and its connective tissue components In rats collagenase does not alter the elastic behaviour but reduces the tensile strength of the lung in contrast to papain and elastase which reduce elastic recoil correlating with the extent of damage to elastic fiber (9)

A therapeutic outlook

Clinical findings in patients with α_1 AT deficiency have focused interest on granulocytes and macrophages and led to rapid expansion of our knowledge on the biochemistry and physiology of endogenous proteases from various sources and their inhibitors Taken together with experiences from emphysema induction in animals with proteases new light has been shed on pathogenetic mechanisms operating in chronic obstructive lung disease Although the evidence linking protease excess (especially elastase) to human emphysema is so far indirect it is obvious that we now have to discuss therapeutic measures Two aspects should be considered the preventive and the symptomatic Studies on a large Swedish series of Pi^{ZZ} individuals collected during 15 years have clearly shown a profound influence of cigarette smoking on prognosis in terms of survival and age at presentation of symptoms (Larsson to be published) Extremely rapid and inexpensive methods are available for mass screening purposes (12) In our opinion screening for homozygosity could be performed at any time before puberty Firm advice on the dangers of smoking and other exogenous agents certainly can improve prognosis considerably in the severe deficiency states In addition it seems highly motivated to intensify research on drugs with potential antielastase activity Umezawa and Aoyagi (28) have isolated peptides from Streptomyces cultures with antielastase activity but at present it is unclear whether they inhibit granulo-

cyte elastase French workers (16) have recently synthesized trifluoroacetylated tripeptides which are potent inhibitors of human granulocyte elastase This and similar compounds may be useful therapeutic agents in diseases in which granulocyte elastase is involved pathogenetically

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BOOK REVIEW

British Medical Bulletin Haemostasis vol 33 no 3 pp 183–291 £5 00 British Council London 1977

This issue of the British Medical Bulletin contains 15 papers covering almost every aspect of recent research on haemostasis

The papers are of high standard and written by well known authorities in the field mainly British authors Four papers are on the platelets and the haemostatic mechanism as a whole Dr Esnouf has written an excellent review of the interactions of the clotting factors and especially on prothrombin activation The biochemistry of fibrinogen and its degradation by plasmin has been surveyed by Dr Gaffney and factor VIII and inherited disorders by Drs Bloom and Peake Both these chapters are really concise lucid and up to date There are also chapters on the physiology of fibrinolysis on normal and abnormal fibrinolysis and on disseminated intravascular coagulation It appears that disseminated intravascular coagulation is still a controversial subject Dr Rizza has written a chapter on the clinical management of haemophilia based mainly on the experience of the Oxford Haemophilia Centre Dr Cash has written on blood replacement therapy in general There is one paper on investigation of a long standing bleeding tendency This survey is too short to be very useful especially concerning acquired chronic defects

As a whole this number gives an excellent review of haemostasis from a biochemical as well as clinical point of view

Inga Marie Nilsson Malmö Sweden

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A Prospective Study of Streptokinase and Heparin in the Treatment of Deep Vein Thrombosis

H Arnesen A Heilo E Jakobsen B Ly and E Skaga

From Medical Department IX and the Department of Radiology Ulleval Hospital University Clinic Oslo Norway

ABSTRACT In a prospective trial 42 medical patients with a history of deep vein thrombosis of less than five days were allocated at random to treatment with streptokinase or heparin Only patients with extensive thromboses were included Streptokinase was given in a loading dose of 250 000 IU and a maintenance dose of 100 000 IU/hour for 4 days as a mean Heparin was given in a loading dose of 15 000 IU and a maintenance dose of 20 000-50 000 IU/day The therapeutic results were evaluated by phlebography Significant thrombolysis occurred in 71 4% of 21 patients treated with streptokinase and in 23 8% of the 21 heparin treated patients Using the χ^2 test for overall association, this difference was statistically highly significant ($p=0\ 002$) Three patients in each treatment group experienced major bleeding two in each group requiring blood transfusions Minor bleeding and slight rise in temperature were encountered more often in the streptokinase than in the heparin group It is concluded that patients with acute deep vein thrombosis with proximal extension of the thrombus beyond the calf veins should be offered a therapeutic trial with streptokinase

Intravenous heparin is the usual therapy in patients with acute deep vein thrombosis in Norway today Since the introduction of fibrinolytic activators like streptokinase several reports on successful treatment of acute deep vein thrombosis have been published (3 5 9 11 12 14 15 18 23) To our knowledge only three studies are prospectively comparing the initial effect of streptokinase and heparin (11 18 23) all of them reporting more extensive and rapid thrombolysis with streptokinase than with heparin

Various dosage schedules of streptokinase have been used in the reported studies To simplify the routine of streptokinase administration we have adopted a standard dosage regimen

The aim of the present study was to evaluate the degree of phlebographically documented thrombolysis in medical patients with acute deep vein thrombosis allocated at random for the treatment with heparin or standard doses of streptokinase

PATIENTS AND METHODS

Selection of patients The study concerned patients with clinical evidence of deep vein thrombosis admitted to the Medical Departments VII VIII and IX at Ullevål Hospital Only patients with symptoms of less than five days were considered and the diagnosis was in all cases confirmed by phlebography Only patients with extensive thrombosis that is with proximal extension beyond the calf veins were included

Patients with known bleeding tendency major surgery within 7 days bleeding from the gastrointestinal or urogenital tract recent cerebrovascular disease arterial hypertension (diastolic pressure 120 mmHg or more or hypertensive retinopathy grade 3-4 (Keith & Wagener)) severe renal or hepatic insufficiency pregnancy or known malignant disease were excluded An arbitrary upper age limit was set to 70 years

When the patients had been admitted to the trial allocation to the treatment groups was performed by using sealed envelopes preformed by our statistician on the basis of random numbers

Phlebographic examination

Method The method used in the present study is similar to methods described by other authors (3 14 20 24)

A rubber tourniquet was placed around the ankle and the contrast medium was injected into a superficial vein on the dorsum of the foot The patient lay in a semi upright position supporting his weight on the contralateral foot To obtain maximal filling of the iliac vein manual pressure was applied to the calf when the examination of that region had been completed

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Braun Melsingen West Germany) The infusion was routinely continued until the control phlebography was performed after 72–90 hours *If complete thrombolysis or no thrombolysis could be documented the streptokinase infusion was discontinued* If partial thrombolysis had taken place the infusion was continued for another 24–48 hours The treatment was given for 96 hours as a mean

When the streptokinase was discontinued oral anti coagulation with warfarin was initiated In addition heparin was given when the thrombin clotting time was less than twice the normal control value Heparin was given as an i v infusion or subcutaneously twice daily to avoid confinement to bed until therapeutic values of Thrombotest were obtained The dose of heparin varied between 10 000 and 30 000 IU/day

To avoid anaphylactic reactions 100 mg of soluble hydrocortisone was given i v before the start of therapy whereafter prednisolone 10 mg 3 times daily was administered orally until discontinuation of streptokinase

Heparin (Apothekernes Laboratorium Oslo Norway) was given in an initial dose of 15 000 IU i v followed by a maintenance dose of 30 000 IU/day given as a continuous i v infusion The dose of heparin was later adjusted according to the thrombin clotting time (final concentration 10 NIH U/ml of thrombin) to maintain a heparin concentration of 0 5–1 0 IU/ml of normal plasma (7) and varied between 20 000 and 50 000 IU/day The infusion was routinely continued until the control phlebography was performed after 72–90 hours Thereafter oral anticoagulation with warfarin was started Heparin was discontinued when therapeutic values of Thrombotest were obtained I m injections were avoided in both treatment groups

Description of the clinical material Forty sealed envelopes were preformed by the statistician All were used during the study One of the patients was later excluded from the study because of a wrong initial interpretation of the phlebogram This patient had a subfascial bleeding in the calf muscles and deteriorated during the first day of streptokinase therapy which was stopped promptly In addition 3 patients had already started specific treatment 1 with heparin and 2 with streptokinase before any envelope was opened Before any evaluation was done they were included in the trial and further treated according to the protocol

The total study population thus comprises 42 patients with the age and sex distribution shown in Table I 21 treated with streptokinase and 21 with heparin Three patients had their control phlebography done after 17–21 days mainly because of technical difficulties at the end of streptokinase (1 patient) or heparin (2 patients) therapy They were excluded from the statistical analyses but otherwise included in the total material

RESULTS

Laboratory assays All patients in the streptokinase group showed extensive systemic fibrinolysis with fibrinogen of 0 2–1 0 g/l and euglobulin lysis time of 5–25 min during therapy

Phlebographic evaluation (Table II) The patients were divided into subclasses according to the phlebographic extension of their thromboses When the thrombus extended from the calf veins up to the iliac vein 4 out of 8 patients in the streptokinase group showed substantial dissolution In addition 2 others had moderate dissolution Thus the effect was reasonably good in 75% of these patients In contrast all 6 patients treated with heparin were unchanged or even worse

A similar pattern was observed in patients with thrombus extension from the calf veins to the femoral vein although 2 of 7 patients in the heparin group showed moderate thrombolysis The tendency to thrombolysis in the heparin group was better demonstrated when the thromboses extended from the calf veins into the popliteal vein only

The rate of resolution was low in the small group of 4 patients with isolated thromboses in the femoral and iliac veins Thus only one of the two patients treated with streptokinase showed moderate thrombolysis The other 3 patients became worse

In the 3 patients in whom control phlebography was performed after 17–23 days 2 with thromboses extending from the calf veins into the iliac vein and treated with heparin were both phlebographically worse when controlled One patient with thrombus extension into the popliteal vein and treated with streptokinase showed substantial dissolution when controlled

In the entire series 71 4% of the streptokinase treated patients showed significant thrombus dissolution (52 4% substantial dissolution 19% moderate dissolution) whereas the corresponding figure in the heparin group was 23 8% (9 5% substantial dissolution 14 3% moderate dissolution) Correspondingly 28 5% of the streptokinase treated patients were unchanged or worse whereas the corresponding figure for the heparin treated patients was 76 3%

All the 4 streptokinase treated patients with phlebographic deterioration had a sufficient systemic fibrinolytic effect as judged from the laboratory analyses In one of them a malignant seminoma of the testis with abdominal metastases was subsequently diagnosed and he died 6 months later The second patient was a 13 year old boy with an appendicitic infiltrate in the small pelvis The third patient had a chronic pyelonephritis with normal renal function She had previous thrombotic

Table III *Clinical evaluation*

	Streptokinase		Heparin	
	n	%	*n*	%
Total	16		14	
Normal	5[a]	31 3	1[b]	7 1
Improved	8	50	9	64 3
Unchanged	2	12 5	4	28 6
Worse	1	6 3	0	

[a] Phlebographically major dissolution in all
[b] Phlebographically unchanged
Phlebographically partial dissolution

episodes in both legs and had also experienced an episode of herpes zoster The fourth of these patients had a chronic bronchitis but no defined thrombogenic disease

Statistical evaluation When the 3 patients in whom control phlebography was performed after 17–21 days were excluded from consideration the statistical analyses using the χ^2 test for overall association (6) showed that thrombolysis was achieved significantly more frequently with strepto- than with heparin The two degrees of mbolysis (substantial and moderate dissolution) analysed together and the *p* value was 0 002 9 55) The χ^2 test for trend (6) showed a *p*-value of 0 012 (χ^2 6 35) The test for differences between various subgroups according to the extension of the thromboses did not indicate that such differences existed (χ^2 1 11 *p* 0 78)

Clinical evaluation The clinical condition on the days of phlebography was used for the present evaluation Based on the measurements of the circumference at three levels (cf Patients and Methods) the clinical status was described as normal improved unchanged or worse in relation to the pretreatment status The evaluation was sufficiently good for analysis in 30 patients 16 in the streptokinase and 14 in the heparin group The results are given in Table III There are some obvious discrepancies with the phlebographic results Thus one patient in the heparin group showed clinical normalization in spite of unchanged phlebography She had proximal extension of the thrombus only into the popliteal vein Only one patient showed clinical deterioration He belonged to the streptokinase group and his control phlebography showed moderate thrombolysis He probably had a haematoma in his calf muscles which would explain the increased circumference of the calf

Moreover the clinical evaluation showed better results than the phlebographic Thus in the heparin group clinical improvement was usually achieved in spite of no demonstrable thrombolysis

Complications (Table IV)

In *the streptokinase group* minor haematomas related to puncture sites were regularly seen One patient exhibited a large haematoma in the groin followed by a drop in Hb from 12 7 to 7 2 g/100 ml requiring transfusion of 1 000 ml of blood A second patient developed a large haematoma in the gluteal region associated with a drop in Hb from 9 6 to 5 8 g/100 ml At this time he also developed a myocardial infarction A blood transfusion of 2 000 ml was given The final result was uncomplicated A third patient developed a pressure haematoma in the gluteal region followed by a drop in Hb from 12 9 to 10 8 g/100 ml Blood transfusion was not given

One patient developed probably pulmonary embolism during therapy documented by lung scan The symptoms normalized rapidly

One patient with pulmonary embolism prior to therapy developed heart tamponade and shock because of a catheter lesion of the coronary sinus during pulmonary angiography on the day after discontinuation of streptokinase Also in this case the late result was uncomplicated

A slight rise in body temperature was more frequently seen in the streptokinase group but no patient developed rigors or other signs of serious streptokinase reaction In no patient did the streptokinase infusion have to be interrupted or stopped because of adverse reactions

In *the heparin group* one patient had haema

Table IV *Complications*

	Streptokinase	Heparin
Major bleeding requiring blood transfusion (no of pats)	2	2
Major bleeding not requiring blood transfusion (no of pats)	1	1
Pulmonary embolism (no of pats)	1	
Other serious complication (no of pats)	1	
Slight rise in body temperature	+	–
Minor haematomas at puncture sites	++	(–)

temesis associated with shock and a drop in Hb from 12 0 to 8 5 g/100 ml A blood transfusion of 1500 ml was given A duodenal ulcer was later diagnosed A second patient developed a retroperitoneal haematoma with shock and a drop in Hb from 14 9 to 9 4 g/100 ml A blood transfusion of 1000 ml was given A third patient experienced severe menorrhagia and slight rectal bleeding followed by a drop in Hb from 12 5 to 8 8 g/100 ml No blood transfusion was given Heparin was discontinued after 15 hours in one patient because of haematemesis with shock Warfarin was given without signs of additional bleeding

Conclusively no patient in this study died and the occurrence of major bleeding was equally frequent in the two treatment groups Minor haematomas and a slight rise in body temperature were seen more often in the streptokinase than in the heparin group Pulmonary embolism was diagnosed once during therapy in a patient in the streptokinase group the symptoms were highly transitory

DISCUSSION

In the present prospective study streptokinase gave significantly better thrombolysis during 4 days treatment than heparin Thus about 70% of the streptokinase treated patients showed phlebographical thrombolysis compared with about 25% of the heparin group This is close to the figures found by Kakkar et al (11) in their prospective study with 9 patients in each treatment group (66 7% and 22 2% respectively) Tsapogas et al (23) found somewhat lower figures for thrombolysis (53% and 7% respectively) in their prospective study with 19 patients in the streptokinase group and 15 in the heparin group They defined thrombolysis as more than 75% resolution of the original thrombus which probably corresponds to substantial thrombolysis in our study and thus fits with our results (52 4% and 9 5% Table II) Duckert et al (5) found that 67% of their 93 patients with deep vein thrombosis and streptokinase treatment showed total or partial thrombolysis documented by phlebography within 14 days after the start of therapy In their heparin group of 42 patients considered unsuitable for thrombolytic treatment only 10% showed phlebographical thrombolysis Kakkar and Flute (12) in their series of 38 streptokinase treated patients with deep vein thrombosis and a history of less than 4 days found 69% total or partial thrombolysis with phlebography performed after 7 days

Thus one can probably expect significant thrombolysis in 70% of streptokinase treated patients with acute deep vein thrombosis With heparin the corresponding figure will be 10–25%

It is well known that the clinical diagnosis of deep vein thrombosis is difficult including false negative and positive diagnoses (21) Phlebography is thought to be the best method for the exact anatomic diagnosis as well as for the evaluation of thrombolysis or thrombotic progress (3 9 17 18 22 23) This was also obvious in our study (Tables II and III) as clinical improvement was more impressive than thrombolysis shown by phlebography The main reason for this discrepancy is probably the use of preexisting collaterals for the venous blood flow The use of superficial collaterals for the venous return will not however hinder the development of the postthrombotic syndrome In addition clinical deterioration occurred in one of four patients in spite of phlebographical thrombolysis We feel that phlebography should be performed for the exact diagnosis of deep venous thrombosis and later as a necessary guide during thrombolytic therapy

Concerning the dose of streptokinase various schedules have been used (3 5 11 12 18 23) Considering the documented results in relation to the doses used only the first series of Robertson et al (18) using only 50 000 IU/hour as a maintenance dose gave negative esults that is no conclusive thrombolysis As almost equal results have been obtained in studies conducted with different dosage schedules and as there is no laboratory assay available to establish optimal thrombolysis we feel that a standard dosage schedule is to be recommended The standard schedule used in the present study with a loading dose of 250 000 IU and a maintenance dose of about 100 000 IU/hour seems suitable If however the patient presents a history of recent streptococcal infection the TID may prove useful for establishing the loading dose (1)

Madar et al (14) and Duckert et al (5) pointed out that thrombi with proximal extension were lysed more easily than thrombi localized more distally only A similar tendency was not seen in our study (Table II) Spontaneous thrombolysis during heparin treatment seemed however to be more readily achieved in cases with distal localization only (Table II) In the light of the two serious com

plications to deep venous thrombosis—massive pulmonary embolism and postthrombotic syndrome—we feel that an arbitrary level in the region of the popliteal vein should be drawn to establish the anatomical indication for streptokinase treatment Thus thrombi with localization in calf veins only should be treated with heparin whereas cases with more proximal extension of the thrombi should be offered a therapeutic trial with streptokinase

Browse et al (3) pointed out that non-occlusive thrombi were lysed more easily than totally occlusive In our study this was not obvious and totally occluding thrombi were lysed as well

In the present study 4 patients had further extension of the thrombus and 2 remained unchanged at the end of streptokinase treatment in spite of satisfactory fibrinolytic activity as judged from the laboratory assays (fibrinogen euglobulin lysis time) The reason for these failures remains obscure The underlying disease was in a majority of these cases chronic or even malignant as diagnosed later The question of the real age of the thrombi also seems relevant in these cases

n the present study minor complications such as slight rise in temperature or small haematomas ated to puncture sites were far more common in the streptokinase treated than among the heparin treated patients Major complications however were equally distributed in the two treatment groups Thus bleeding requiring blood transfusion appeared in 2 patients in each group (9 5%)

No patient died in the present study We feel that the frequency of serious complications was of the same magnitude in the two treatment groups The bleeding tendency seen during streptokinase treatment should in our opinion not hinder the common use of this drug in acute deep vein thrombosis provided the patients are carefully screened beforehand for other bleeding tendencies arterial hypertension and malignant disease

The exact advantage of streptokinase therapy cannot be established until the ultimate fate of the thrombosed veins is known Thus the frequency with which maintained valvular function and avoidance of the postthrombotic syndrome can be achieved is not yet settled Optimistic results have nevertheless been obtained by Kakkar et al (13) in 1969 and recently by Bieger et al (2) and Johansson et al (10)

With present knowledge it seems reasonable to advocate streptokinase as the best treatment in cases of acute deep vein thrombosis of the leg with proximal extension of the thrombus into the popliteal vein A careful screening for bleeding tendencies should be carried out before initiation of treatment A standard dosage schedule seems suitable and simplifies the practical use of streptokinase

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A Controlled Clinical Trial of Streptokinase and Heparin in the Treatment of Major Pulmonary Embolism

B Ly H Arnesen H Eie and R Hol

From Medical Department IX and the Department of Radiology Ullevål Hospital University Clinic Oslo Norway

ABSTRACT Treatment with streptokinase or heparin was allocated randomly to 20 patients with major pulmonary embolism verified by angiography In addition 4 patients treated with streptokinase and 1 patient treated with heparin were included in the trial prior to the start of treatment Streptokinase or heparin was given for 72 hours and pulmonary angiography was repeated The angiographic evidence of thrombolysis was significantly greater ($p<0.01$) in the 14 patients treated with streptokinase than in the 11 treated with heparin In the heparin group 1 patient died from massive embolism 15 hours after the start of treatment In another patient who died 4 weeks later from cerebral glibolastoma persistent massive embolism contributed to the fatal outcome In the streptokinase group, 1 patient with a metastatic pulmonary carcinoma died 3 weeks after the start of treatment from gangrene of both legs following thrombotic occlusion of the inferior vena cava Bleeding was more common after treatment with streptokinase than with heparin but was not a serious problem in any patient It is concluded that patients with life threatening pulmonary embolism should be offered the benefits of streptokinase

Pulmonary embolism is a common disease in medical and surgical patients Although most patients recover spontaneously or die before treatment can be given (15) a small proportion are severely ill and some die several hours or days following the embolic episode A number of reports claim that fibrinolytic treatment with streptokinase or urokinase is superior to heparin in such patients (6 8 10 16)

However to our knowledge only two randomized controlled trials are prospectively comparing the effect of fibrinolytic treatment and heparin in patients with major pulmonary embolism (12 18) In the Urokinase pulmonary embolism trial (12) urokinase when compared with heparin significantly accelerated the resolution rate of pulmonary thromboemboli at 24 hours In the trial performed by Tibutt et al (18) significantly greater evidence of thrombolysis was reported in patients treated with streptokinase for 72 hours than in patients given heparin for the same period It is not yet settled however whether the increased rate of thrombolysis following fibrinolytic treatment reduces the mortality in patients with massive pulmonary embolism

This report concerns a controlled trial in which medical patients with proven major pulmonary embolism were allocated at random to treatment with heparin or a standard dose of streptokinase

STUDY POPULATION AND METHODS

Selection of patients Any patient admitted to one of the medical departments Ullevål Hospital with symptoms of acute major pulmonary embolism was considered for the trial Only patients with symptoms of less than five days were included and the diagnosis was in all cases confirmed by angiography Patients with minor embolism affecting less than one lobar artery were excluded Excluded were also the following categories Patients with known bleeding tendency or recent bleeding from the gastrointestinal or urogenital tract major surgery within the last 10 days recent cerebrovascular episodes severe hypertension (diastolic pressure 120 mmHg or more or hypertensive retinopathy grade 3–4) severe renal or hepatic insufficiency pregnancy recent delivery or known malignant disease An arbitrary upper age limit was set to 70 years Allocation of patients to the two treatment groups 14 in the streptokinase 11 in the heparin group was performed

using sealed envelopes on the basis of random numbers

Pulmonary angiography Selective bilateral pulmonary angiography has been the procedure of choice In some instances however non selective pulmonary angiography was performed Angiography was done in the acute stage on admission and repeated for control after 3–4 days of treatment (after 72 hours as a mean)

Interpretation of pulmonary angiographs All pulmonary angiographs were reviewed independently by two radiologists who had no knowledge of the clinical state of the patients or the treatment given The severity of the pulmonary artery occlusion was assessed by the score system devised by Miller et al (10) This system provides a score from zero to 34 made up of a maximum of 16 for the thrombus itself and 18 for peripheral perfusion as assessed by opa ification of the peripheral vessels by contrast medium

Pretreatment evaluation The history and clinical features were recorded and a 12 lead ECG and a chest X ray were taken of all patients In patients with less severe symptoms lung scanning (gamma camera) was performed prior to pulmonary angiography Venous blood was drawn for the following estimations Hb thrombocyte count Thrombotest activated partial thromboplastin time fibrinogen (the method of Clauss (2)) thrombin clotting time (final concentration of 1 NIH U/ml of thrombin) euglobulin lysis time (13) ethanol gelation test (7) serum creatinine S GOT and S GPT Except for thrombocyte count partial thromboplastin time and serum creatinine the same estimations were performed daily during treatment

Clinical evaluation during treatment Clinical features ted daily during the first 10 days after the start of nent as was any sign of bleeding When possible a ily ECG was recorded Special care was taken to record any sign of deep vein thrombosis or recurrent pulmonary embolism

Treatment schedules

Streptokinase Streptokinase (Streptase Behringwerke Marburg Lahn West Germany) supplied by Norske Hoechst was given in standard doses After a loading dose of 250000 IU dissolved in 20 ml 0 9% NaCl given i v in 20 min a maintenance dose of 100000 IU/hour was given by continuous i v infusion (Infusomat Braun Meslingen West Germany) The infusion was continued until control pulmonary angiography after 72 hours Streptokinase was then discontinued and oral anticoagulation with warfarin was started In addition when the thrombin clotting time was less than twice the normal control value heparin was given i v in doses of 10000–30000 IU/day until therapeutic values of Thrombotest were obtained

To avoid anaphylactic reactions 100 mg of soluble hydrocortisone were given i v before the loading dose of streptokinase whereafter prednisone 10 mg 3 times daily was given until discontinuation of streptokinase

Heparin Heparin (Apotekernes Laboratorium Oslo Norway) was given in an initial dose of 15000 IU i v followed by a maintenance dose of 30000 IU/day as a continuous i v infusion The dose of heparin was subsequently adjusted according to the thrombin clotting time

Table I *Pretreatment clinical data*

Age heart rate *BP Hb* and fibrinogen are given as mean values with range in parentheses

	Heparin	Streptokinase
No of pats	11	14
Males females	3 8	8 6
Age (y)	56 (23–70)	51 (37–68)
Surgery or trauma in preceding 4 weeks	4	5
Cardiac disease	1	1
Malignant disease diagnosed later	1[a]	1[b]
Oral contraception		1
Previous deep vein thrombosis	5	6
Varices	4	1
Previous pulmonary embolism	2	3
Clinical symptoms		
Syncope collapse	1	1
Chest pain (pleural)	8	12
Chest pain (central)	2	1
Dyspnea	8	13
Hemoptysis	1	3
Fever (>38 C)	7	8
Heart rate/min	105 (80–140)	90 (76–104)
BP (mmHg)		
Systolic	130 (80–170)	130 (80–155)
Diastolic	83 (60–100)	79 (70–95)
Signs of deep vein thrombosis on admission	4	7
Hours from onset of symptoms to initiation of treatment		
0–24	3	5
24–48	4	6
48–72	4	1
72–96	0	0
>96	0	2
ECG		
Normal	8	8
S_1 Q_3 T_3 or Q_3 T_3 pattern	3	3
ST depressions or negative T in		
Right precordial reading	3	3
Left precordial reading	2	3
Hb (g/100 ml)	13 4 (9 5–15 3)	12 2 (9 7–14 7)
Fibrinogen (g/l)	4 38 (2 95–700)	4 30 (1 85–7 00)
Ethanol gelation test		
Positive	9	5
Negative	2	9
Angiographic score (mean ±1 S D)	18 1±8 0	21 6±6 6

[a] Cerebral glibolastoma [b] pulmonary carcinoma

Table II *Angiographic score (mean±1 S D) before and after 72 hours treatment*

	Heparin (10 pats)*	Streptokinase (14 pats)	
Before treatment	16 5±6 3	21 6±6 6	
After treatment	13 1±7 6	10 2±6 7	
Improvement	3 4±6 8	11 3±5 7	$p<0\ 01$

The patient who failed to complete the 72 hour trial has been excluded

(final concentration of 10 NIH U/ml of thrombin) to maintain an in vivo heparin effect corresponding to a heparin concentration of 0 5–1 0 IU/ml in normal plasma The daily dose thus varied from 30000 to 60000 IU Warfarin was started after control angiography and heparin was discontinued when therapeutic values of Thrombotest were obtained As a mean heparin was given for 7 days but was continued for 17 days in one patient with symptoms of persistent massive embolism

I m injections were avoided in both treatment groups

Description of the clinical material Twenty five patients entered the trial and all except 5 were allocated to treatment groups by using sealed envelopes Four of these 5 patients were treated with streptokinase and one with heparin The decision to include these 5 patients in the trial was made prior to the start of treatment These patients were not allocated at random for the following reasons Of the 4 streptokinase treated patients one seriously ill patient with massive embolism (angiographic score 24) was transferred from another hospital for fibrinolytic treatment Another seriously ill patient (angiographic score 25 mean pulmonary arterial pressure 35 5 mmHg arterial blood pO_2 50 5 mmHg) was considered a candidate for pulmonary embolectomy but it was decided to treat him with streptokinase One patient had a history of 10 days (angiographic score 24) and one probably had the first of two embolic episodes 3 weeks earlier (angiographic score 16) The heparin treated patient was not allocated at random because of ulcerative colitis (angiographic score 20)

The clinical features were similar in both treatment groups except that the mean age in the heparin group was somewhat higher and that there were more women than men in the heparin group and vice versa in the streptokinase group (Table I)

Only one patient in each group had a systolic BP below 90 mmHg and 3 patients in each group had ECG signs of acute right ventricular strain (Table I) The interval from onset of symptoms to initiation of treatment was similar in both groups except that 2 patients in the streptokinase group had a history of more than 5 days (not allocated randomly)

The illness was somewhat severer as judged by angiographic scores in the streptokinase than in the heparin group (Table I) There was however only one patient in each group with an angiographic score of more than 30 and these patients were both in shock on admission

RESULTS

Response to treatment

The changes in angiographic score after 72 hours of treatment are shown in Table II The two treatment groups showed significantly different improvement in angiographic score as evaluated by Student s *t* test ($p<0\ 01$) The mean angiographic score fell 52.3% in the streptokinase group but only 20 6% in the heparin group When the 5 patients not randomly allocated were excluded the mean improvements in angiographic scores were 3 7±7 2 (heparin group) and 10 3±5 1 (streptokinase group) This difference was also significant ($p<0\ 05$)

Of the 10 patients treated with streptokinase and who had an initial angiographic score of more than 20 8 had a score of less than 16 at 72 hours In contrast the corresponding figures in the heparin group were 2 out of 6 patients while the score remained unchanged in another 2 In addition angiographic deterioration (rise in score from 15 to 24) occurred in one patient treated with heparin After discontinuation of heparin this patient experienced clinical and angiographic improvement (fall in score from 24 to 12) following treatment with streptokinase for 3 days

The clinical progress in relation to the angio-

Table III *Clinical versus angiographic response to 72 hours treatment in patients with angiographic scores above 20*

	Streptokinase (10 pats)	Heparin (6 pats)
Marked clinical and angiographic improvement (no of pats)	8	2
Difference in score (mean)	13 5	14
Clinical improvement angiographic appearance slightly improved unchanged or worse (no of pats)	1	1
Difference in score	3	0
No clinical improvement angiographic appearance slightly improved unchanged or worse (no of pats)	1	2
Difference in score	4	0 −9
Clinical deterioration treatment failure (no of pats)		1 (died after 15 h)
Initial score		34

Table IV *Side effects of treatment*

	Heparin	Streptokinase
Major bleeding requiring blood transfusion	1	2
Major bleeding not requiring blood transfusion	1	2
Hb fall (>1 g/100 ml)	2	4
Rise of temperature (>1 C)		2
Site of bleeding		
Puncture or cut-down sites	1	3
Operation sites		1 (melena)
Hematothorax		1
Hematuria	3	

graphic evidence of thrombolysis in patients with angiographic scores above 20 is shown in Table III The clinical progress during the treatment period of 72 hours was satisfactory in all patients with marked angiographic improvement regardless of the type of treatment It should be noted however that 8 of the 10 patients in this category had been treated with streptokinase It is also important to note that clinical improvement might occur in spite of unchanged or even worse angiographic appearance

J that the clinical features might be moderate in

with massive embolism On the other hand

of the 3 patients with an unsatisfactory clinical progress (one treated with streptokinase and two with heparin) had obtained s gnificant thrombolysis as judged by angiography

Treatment failures deaths

One patient in the heparin group a 66-year-old woman failed to complete the 72 hour trial She presented with severe hypotension and an angiographic score of 34 deteriorated and died 15 hours after starting heparin treatment Necropsy demonstrated large occluding thrombi in both the right and the left pulmonary artery

The other heparin treated patient who died was a 49 year-old man Five weeks prior to embolization he was operated upon because of a tumor located in the right frontal lobe of the cerebrum and the histological diagnosis was meningioma He was transferred to the Medical Department with the clinical features of severe dyspnea and cyanosis and was also moderately hypotensive Angiography demonstrated central emboli on both sides with no change following 72 hours treatment with heparin (angiographic score 24) The heparin infusion was continued for 17 days and there was only a slight clinical improvement but later he became mentally confused and subsequently died 13 days after discontinuation of heparin Necropsy revealed multiple partially occluding and organized emboli in several lobar arteries A glioblastoma was found in the right hemisphere As judged from the clinical course persistent embolism contributed to the fatal outcome

In the streptokinase group there was one late death a 66-year-old woman with an undiagnosed pulmonary adenocarcinoma She had a one week history of deep vein thrombosis of the right leg and had complained of dyspnea during the last two days Phlebography demonstrated deep vein thrombosis on both sides probably affecting the iliac veins Bilateral major pulmonary embolism was shown by angiography and the angiographic appearance was only slightly improved following streptokinase The patient died 3 weeks later from phlegmasia coerulea dolens with gangrene of both feet Necropsy disclosed occluding thrombi in vena cava inferior and in the iliacal and femoral veins on both sides but no evidence of thrombotic occlusion of the arteries Pulmonary emboli could not be demonstrated An undifferentiated adenocarcinoma was found in the right lung and metastases were demonstrated in the pancreas the kidneys and the adrenals

Side effects of treatment

As shown in Table IV bleeding was more frequent in the streptokinase than in the heparin group Thus 4 streptokinase treated patients had major bleedings compared to only 2 in the heparin group It is important to note however that bleeding in the streptokinase group was most often related to puncture or cut-down sites and that spontaneous bleeding was not more common than in patients treated with heparin A rise in temperature was more frequent in the streptokinase group but more serious anaphylactic reactions were not seen In no case did streptokinase have to be withdrawn because of side effects

DISCUSSION

Numerous case reports have indicated that in man substantial resolution of pulmonary embolism may occur spontaneously within days or weeks as demonstrated by pulmonary angiography (5 17) However the early resolution rate of major pulmo-

nary emboli is slow as judged by angiography or lung scanning (1 4 19) and significant resolution of major emboli does not seem to occur during the first week

Contrasting with these reports of slow resolution of pulmonary embolism during treatment with heparin are reports of rapid lyis after streptokinase or urokinase (6 8 10 12 16 18) When comparing the effects of heparin and streptokinase in pulmonary embolism it is a prerequisite that the patients in each group are comparable with respect to prognostic factors and factors known to affect resolution Such factors are the severity and duration of the embolism and coexisting cardiorespiratory disease The present treatment groups were comparable except that the angiographic obstruction was severer in the streptokinase than in the heparin group and that the duration of the embolism was longer than 1 week in two streptokinase treated patients This however would be expected to disfavour the spontaneous thrombolysis in the streptokinase group (6 17 19) As shown the angiographic evidence of thrombolysis was significantly greater ($p<0.01$) in the 14 patients treated with streptokinase than in the 11 treated with heparin The mean angiographic score thus fell by 52 3% in the streptokinase group and by 20 6% in the heparin group which agrees reasonably well with the respective values of 61% and 15% obtained by Tibutt et al (18)

For practical reasons right sided pressure measurements were performed in only 4 of our streptokinase treated patients of whom 3 had normal pulmonary arterial pressures following streptokinase This finding is in accordance with the observation made by others (6 8 12 18) that the enhanced thrombolysis obtained with fibrinolytic treatment is accompanied by a reduction of systolic and mean pulmonary arterial pressures One patient deteriorated during treatment with heparin but was subsequently successfully treated with streptokinase thus serving as his own control

The systemic fibrinolytic effect was sufficient in all streptokinase treated patients as judged from the coagulation assays Hence the failure to obtain significant thrombolysis in 2 of the streptokinase treated patients remains obscure The question of the real age of the thrombi might be relevant in these patients

In the present study only 1 patient being treated with heparin suffered early death from massive obstruction of the pulmonary artery She had severe hypotension and an angiographic score of 34 According to the prognostic criteria outlined by Kakkar and Raftery (9) and later stressed by Tibutt et al (18) her only chance of survival would probably have been embolectomy Strict indications for embolectomy are however difficult to decide Thus in the present study a critically ill patient with severe hypotension and an angiographic score of 31 was successfully treated with streptokinase

The important question of whether the accelerated thrombolysis obtained with fibrinolytic treatment might reduce mortality in massive pulmonary embolism is not yet settled As stressed by Tibutt et al (18) a trial including much larger numbers of subjects than those published until now would be needed for this

The possible benefits of streptokinase in major pulmonary embolism must be weighed against possible harmful side effects In the present study major bleeding was more frequent in streptokinase treated patients than in those treated with heparin which accords with some reports (6 8 12) but not with others (10 18) It is important to note that the higher frequency of bleeding in the streptokinase group was related to puncture or cut down sites in connection with diagnostic procedures Three patients developed inguinal hematoma following pulmonary angiography To avoid this complication catheterization for pulmonary angiography in patients with suspected pulmonary embolism should be performed via the medical cubital vein which is to be ligated prior to treatment with streptokinase

In patients with acute life threatening pulmonary embolism one has to make the choice between three types of treatment heparin streptokinase or emergency embolectomy Pulmonary embolectomy with cardiopulmonary bypass still carries a significant mortality though this has been gradually reduced in recent years (3 14 20) According to Tibutt et al (18) embolectomy should be restricted to patients with sustained hypotension and an initial angiographic score of 24 or more

Otherwise we feel that streptokinase is the treatment of choice for most patients with acute life threatening pulmonary embolism provided that a careful screening for bleeding tendencies is carried out before treatment is started It is also important that the number of invasive diagnostic procedures should be kept as low as possible

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Activated F IX Concentrate (FEIBA) Used in the Treatment of Haemophilic Patients with Antibody to F VIII

S Stenbjerg and J Jørgensen

From the Blood Bank Blood Grouping Laboratory and Tissue Typing Laboratory Department of Rheumatology and Physical Medicine and Department of Pediatrics Århus Kommunehospital University of Århus Århus Denmark

ABSTRACT Bleeding episodes in five haemophiliacs with antibody to F VIII were treated by activated F IX concentrate (FEIBA) Relief of pain and haemostasis in affected muscles and joints were recorded in each case One patient developed a mild attack of disseminated intravascular coagulation with an uneventful recovery A substantial rise in natural inhibitors of coagulation was seen in two patients, and all but one experienced a rise in F VIII antibody titer

Bleeding episodes in haemophilic patients with an antibody to F VIII present severe problems to the patients and the clinician since no therapeutic approach has been generally agreed upon Low titer antibodies may be overcome temporarily by massive doses of F VIII with simultaneous administration of cytostatic drugs in order to delay the secondary immune response to F VIII (8) while extensive plasmapheresis and huge amounts of F VIII have been tried in patients with high titer antibodies (9)

A new approach involves the use of F IX concentrates with trace amounts of activated coagulation factors the nature of which is still obscure (6) These activated substances are believed to bypass the antibody possibly by direct activation of F IX or F X There is a potential risk that the F IX concentrate may induce a hypercoagulable state since thromboembolic complications have been observed in haemophilic patients (5) Reports on the use of activated concentrates in haemophilic patients are still scarce (2 3 10 13) and only one case of suspected disseminated intravascular coagulation has been published (12)

The present work summarizes the clinical and laboratory experiences with a new activated factor IX concentrate (FEIBA) used in five haemophilic patients with antibody to F VIII

METHODS

Venous blood (1 part of 3 8 % sodium citrate+9 parts of blood) was obtained prior to and one hour after each FEIBA infusion Platelet poor plasma was frozen at −60°C until assayed Whole blood coagulation time (WBCT) activated partial thromboplastin time (APTT) (Cephotest Nyegaard Oslo) Quick s prothrombin time plasma thrombin time (PT) fibrinogen level fibrinogen degradation products (FDP) ethanol gelation test and thrombocyte count were performed as described earlier (11) Antithrombin III was quantitated by rocket immune electrophoresis with antibody obtained from Nyegaard Oslo

Anti F Xa was assayed by the method of Biggs et al (1) Total antithrombin activity (TATA) was assayed by the method of Innerfield (4) F VIII antibody was assayed by incubating dilutions of the patient s plasma with a F VIII standard (cryoprecipitate) for two hours at 37°C Residual F VIII activity was measured in a one stage technique (7) and the result was calculated so that 1 U of antibody neutralized the F VIII activity in 1 ml of normal plasma

FEIBA (factor eight inhibitor bypassing activity) an activated F IX concentrate was supplied by Immuno Vienna The producer claims that this product shortens the WBCT and to some extent the APTT in patients with antibody to F VIII by mechanisms not yet known The producer has quantitated the FEIBA activity by its in vitro ability to shorten the APTT of a standard F VIII antibody plasma The in vivo effect of FEIBA on the WBCT is related to the strength of the F VIII antibody It is therefore recommended to raise the dose of FEIBA in the event of a rising antibody titer

Table I *Coagulation studies before (B) and one hour after (A) FEIBA treatment (cases 1–5)*

WBCT=whole blood coagulation time APTT=activated partial thromboplastin time Quick=Quick s prothrombin time PT=plasma thrombin time FDP=fibrinogen degradation products n=negative p=positive

Treatment period	Day	FEIBA (U/kg)	WBCT (<5 min)		APTT (<35 sec)		Quick (<17 sec)		PT (<30 sec)		Platelets (200–600 $\times 10^9$/l)		FDP (<10 mg/l)		Ethanol gelation test		F VIII antibody (U/ml)
			B	A	B	A	B	A	B	A	B	A	B	A	B	A	
Case 1																	
I	1	15	43	16	100	75	17	14	29	28	431	436	20	20	n	n	29
	2	20	31	15	94	73	16	14	24	24	400	397	40	40	n	n	
	3	25	32	17	80	79	15	16	22	25	345	361	20	20	n	n	
	4	30	19	15	82	71	16	15	28	28	366	388	20	20	n	n	
	7	50	21	18		73		14		29		385		20	n	n	
	8	50	23	15	76	70	16	14	27	32	343	374	20	20	n	n	
	10	25	25	19	72	73	18	14	25	25	392	363	20	20	n	n	
	11	50	24	19	74	71	17	14	23	24	346	327	20	20	n	n	
	14	50	26	26	80	76	18	16	22	22	328	344	20	40	n	n	26
	15	*75*	*26*	*27*	*86*	*77*	*16*	*16*	*29*	*28*	*312*	*286*	*0*	*0*	*n*	*n*	
II	1	120	29	25	104	91	16	15	24	24	399	423	5		n	n	29
	2	120		27	92		16	14	24	28	320	425			n	n	
	3	120	38	28	95	86	16	14	26	27	248	250			n	n	
Case 2																	
	1	50	44	30	90	80	14	13	22	22	499	503		5	n	n	20
	2	100	34	25	87	77	15	13	22	27	448	455	10	5	n	n	
	3	185	32	19	84	76	14	13	22	23	511	405	5	5	n	n	
	4	185	22	17	81	75	15	14	21	23	418	436	5	10	n	n	
	5	185	24	18	88	70	14	14	24	28	445	366	5	10	n	n	
	6		19		86		15		24		433		5		n		
	7		23														
	27																41
Case 3																	
	1		39		99		17		23		486		30		n		8
	2	30*	39	29		87		14		23	390			15		n	
	5	60*	39	30	89	77	17	14	27	28	357	256	30	480		n	
	6		37		86		17		25		340		3 840		p		
	7		45		98		17		28		350		30		p		
	8		47		99		18		28		387		30				
	12		45		104		17		28		417		60		n		112
Case 4																	
I	1	15	40	27	67		17		26		465	441			n		3
	2	60	24	13							498						
II	1	60	50	34	112	99	16	16	24	25	450	456	10	5	n	n	40
	2	120	44	22	94	79	16	16			443	456	40	5	n	n	34
III	1	120		25		71		15		25		436		5		n	
	2	60	27	21		80		13		26	480	458		10		n	
	3	100	34	18		84		12		26	463	470		5		n	
	4	100		23		65											
IV	1	100		29		86		14		28		439		5		n	
	2	100	42	18	97	81	15	14	22	23	397	388	5	5	n	n	
	3	100	29	19	90		15		29		390						
	4	100	27														
	5	100	45	16			15		33		439						36
Case 5																	
I	1	15	34	29	85	83	16	14	24	19	425	416		20	n	n	
	1	30		26		75		14		24		450				n	
	2	70	29	21	81	77	16	15	25	26	436		20	5	n	n	1 4
	3	80	31	25		65		14		20		409				n	

* Simultaneous administration of 800 U cryoprecipitate

Table I *Continued*

Treatment period	Day	FEIBA (U/kg)	WBCT (<5 min) B	WBCT A	APTT (<35 sec) B	APTT A	Quick (<17 sec) B	Quick A	PT (<30 sec) B	PT A	Platelets (200–600 ×10⁹/l) B	Platelets A	FDP (<10 mg/l) B	FDP A	Ethanol gelation test B	Ethanol A	F VIII antibody (U/ml)
	4	110	27	20		70		13		25		460				n	
	5	110	26	23		71		14		24		415	10	20		n	
	6	70	34	24	83	75	18	14	28	25		496				n	
	7	140		32		79		14		26		391		20		n	
	8	140	25	19		74		14		26		420		10		n	
	9	70	22	24		74		14		25		496		0		n	
	10	70	19	20		75		14		27		448		5		n	
	11		24														
	12		25		89		17		29		469		10		n		
	13		38														
	19		45														
II	1	70	40	33	94	96	17	15	32	29	410	296	5	5	n	n	120
	2		39														
	3		34														
	4		31		97		16		25								

CASE REPORTS

Case 1

A 10-year-old haemophilic boy (F VIII activity <1% previously without antibody to F VIII) who had received numerous infusions of cryoprecipitate developed a large haematoma of the cheek After two weeks of F VIII infusions (cryoprecipitate) the patient developed a potent antibody to F VIII (29 U/ml) FEIBA was administered starting with 15 FEIBA U/kg and rising to 75 U/kg twice daily without ill effect Initially the WBCT shortened from 45 to 11 min (25 FEIBA U/kg) and rose subsequently to 25 min in spite of increased FEIBA doses (75 U/kg)

When the infusions had been terminated the haematoma resolved slowly over six weeks leaving a slight indentation Four months later the patient suffered a large bleeding in the muscles of the right thigh FEIBA (60–100 U/kg) was administered during three days with little effect on the WBCT while the clinical effect was encouraging relief of pain on day two and reduced circumference of affected limb on day three and onward The patient was discharged on day six and all traces of the haematoma had resolved when he was seen on day 15

Case 2

A 20-year-old haemophilic man who developed a F VIII antibody (10 U/ml) several years ago On admission the patient had a haematoma covering introitus laryngis so that inspection of the vocal cords was impossible His voice was hoarse and he complained of dysphagia

FEIBA (50–185 U/kg) was administered during 5 days The WBCT dropped from 44 min to a minimum of 17 min The haematoma decreased in size and the voice became normal within 5 days of treatment The patient was discharged within a week and has not presented himself for control

Case 3

A 20-year-old haemophilic man in whom a F VIII antibody was diagnosed several years ago During the last 8 years he had received no infusions and had suffered only minor bleedings At present he developed a severe haemorrhage in a knee and FEIBA treatment was undertaken at 30 U/kg on day one and 60 U/kg on day two Cryoprecipitate (800 U) was administered simultaneously On the second day of treatment the patient complained of severe headache and general malaise immediately after infusion of FEIBA and cryoprecipitate The coagulation tests indicated a mild attack of disseminated intravascular coagulation No further treatment was given and the coagulation tests returned to normal within two days Liver function tests revealed a slight rise in alkaline phosphatase On day six the swelling and the pain in the knee had almost disappeared and the patient was sent home This case has been published elsewhere (12)

Case 4

A 5 year old haemophilic boy who developed a low titer F VIII antibody (3 U/ml) one year prior to FEIBA treatment The patient had recurrent bleedings in one knee and FEIBA infusions were undertaken to support physical training of the knee joint Rehabilitation was started with one daily infusion of 15–60 U/kg During FEIBA therapy physical training was possible without complicating haemorrhage

One month later the patient suffered a bleeding in an elbow and FEIBA (60 U/kg) was given with 500 U of cryoprecipitate On the following day the pain had disappeared and the joint functioned almost normally Un

Table II *Antithrombin III (AT III) anti F Xa and total antithrombin activity (TATA) determined in cases 1 and 5 during and after FEIBA therapy*

	Case 1			Case 5		
Day	AT III (66–111%)	Anti F Xa (70–140%)	TATA (50–140%)	AT III (66–111%)	Anti F Xa (70–140%)	TATA (50–140%)
Treatment period I						
1		109				
6					81	146
10	92	109				
12					81	270
15	84					
17			173			
21		135				
24	155	140				
32			400			
Treatment period II						
1				94/86	73/78	93/174
2				89		150
3				94		106
4				94	75	115

fortunately it was not possible to obtain blood samples for coagulation studies in this instance

Six weeks later the patient bled in an ankle and FEIBA treatment was started with 60 U/kg followed by 120 U/kg on day two since the initial dose shortened the WBCT to 34 min The F VIII antibody titre had risen from 3 to U/ml since the FEIBA treatment and cryoprecipitate fusions two months earlier On day four the swelling of the ankle had disappeared and the function had normalized

Two weeks later the patient bled in the left knee which became distended and painful FEIBA was infused at 120 U/kg on day one 60 U/kg on day two and 100 U/kg on day three The swelling decreased from day two onwards and the patient was discharged on day four Three days later he presented a bleeding in an elbow and received FEIBA 100 U/kg There was a fast relief of pain and the swelling diminished over 24 hours

One month later a new bleeding appeared in the left elbow with paresis of the forearm muscles FEIBA was given for five days at 100 U/kg with regression of symptoms from day three The patient received another 100 U/kg of FEIBA during the following week because of haemorrhage in an ankle By that time there was almost full restoration of muscle function in the left forearm and hand

Case 5

A 20-year-old haemophilic man with antibody to F VIII diagnosed several years ago The patient developed a *large haematoma in his left calf* after a slight trauma The skin was distended bluish and on the verge of bursting

Treatment with FEIBA was started at 15 U/kg with minimal effect on the WBCT *The dose was increased over 5 days to 150 U/kg* which brought the *WBCT from 35* min to a minimum of 18 min one hour after infusion The coagulation status seemed to be in a steady state after two days of FEIBA treatment (150 U/kg) since it was possible *to reduce the FEIBA dose to 75 U/kg* without prolongation of the WBCT On day 10 a red confluent exanthema was observed and FEIBA treatment was terminated From day six the circumference of the affected leg gradually diminished and after two ultrasonic treatments the fluctuation was negligible

The patient was discharged on day 20 FEIBA treatment *was attempted again 2 weeks later* but a general rash developed a few minutes after start of infusion The F VIII antibody had risen considerably after the first FEIBA treatment (1 4–120 U/ml)

RESULTS

The laboratory findings before and after FEIBA therapy are presented in Table I A constant finding was the *shortening of WBCT even after low* initial doses of FEIBA In four of the five patients FEIBA infusions brought down the WBCT to values between 10 and 20 min It was anticipated that a *further rise in FEIBA concentrations* might carry risks of thromboembolic complications The APTT was less influenced by the presence of FEIBA and there was often a discrepancy between the effect on the WBCT and the APTT *The Quick s prothrombin time* shortened by a few seconds after FEIBA infusions while the PT showed no uniform changes The platelet count was generally unaffected and the ethanol gelation test remained negative

Activation of the fibrinolytic system was not detected except in case 3 (Table I) The patient developed clinical and laboratory signs of a hypercoagulable state after the second day of treatment although the shortening of the WBCT in his case was minimal with values far from the normal range In this case platelet count dropped immediately by about 30% and returned to pretreatment values within 24 hours while the ethanol test remained positive for two days Raised FDP values were noticed for several days A drop in the platelet count was seen in other instances case 2 (Table I days 3 and 5) after FEIBA infusions of 185 U/kg and case 5 (Table I period II day 1) after 70 U/kg However no other parameters indicated a hypercoagulable state

In case 1 (Table I) the effect on the WBCT diminished from the eighth day of treatment in spite of rising FEIBA doses An attempt to elucidate this finding included the determination of antithrombin III values and anti F Xa values (published in detail elsewhere (13))

A rise in natural inhibitors of coagulation was found (Table II) A similar resistance was suspected during the first day of treatment in case 5 (Tables I and II) Antithrombin III anti F Xa and TATA were estimated several times before and after the last FEIBA treatment in this case A substantial rise in inhibitors was recorded (Table II) A rise in F VIII antibody was noticed in two patients (cases 3 and 4) who received FEIBA and cryoprecipitate and in another two cases (2 and 5) who did not receive cryoprecipitate The resistance to FEIBA activity in case 1 was not accompanied by a rise in F VIII antibody level

DISCUSSION

FEIBA was used to treat bleeding episodes in five haemophilic patients with F VIII antibody FEIBA doses ranged from 15 to 185 U/kg b wt and an initial shortening of the WBCT was recorded in all patients It was our impression that the FEIBA treatment was followed by relief of pain and an accelerated decrease of swelling of the involved muscles and joints This seemed to indicate that no further bleeding occurred

In one patient (case 1) a gradually rising in vitro resistance to FEIBA was noticed and a similar complication was suspected in case 5 This in vitro finding was partially explained by a rise in natural inhibitors of coagulation A prompt and substantial rise in TATA was the most striking feature

A serious side effect of FEIBA treatment was the rise in F VIII antibody in four of the five patients however in cases 3 and 4 one cannot rule out the possibility that the addition of cryoprecipitate treatment was responsible for this Disseminated intravascular coagulation the most dreaded side effect of activated F IX concentrate was diagnosed in one patient (case 3) who received simultaneous infusion of cryoprecipitate The attack was mild and the recovery uneventful

FEIBA was administered by English et al (2) in doses of 30–75 U/kg three times daily to control bleeding in a patient with F VIII antibody and traumatic liver rupture They observed a prompt correction of WBCT and the bleeding was controlled within a few days when antifibrinolytic therapy (tranexamic acid) was added No ill effects of FEIBA were recorded On the other hand Pollock and Lewis (10) were unable to correct the WBCT in their patient with FEIBA doses of 80 U/kg and bleeding from a bone fracture did not diminish after treatment The dose of FEIBA used in this case was probably too low since we found it necessary to infuse 185 U/kg in a patient with a similar F VIII antibody level (20–40 U/kg case 2)

It is our overall impression that FEIBA treatment is a therapeutical approach that should be considered in patients with antibody to F VIII since we observed a beneficial clinical effect in all our patients The side effects are however to be kept in mind especially the rise in antibody titer in some patients At present such high responders cannot be identified in advance Reports on the use of FEIBA are still scarce and we should like to urge other centers to publish their experience with activated concentrates for a final evaluation of such products

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A Family with Thromboembolic Disease Associated with Deficient Fibrinolytic Activity in Vessel Wall

Lilian Johansson Ulla Hedner and Inga Marie Nilsson

From the Coagulation Laboratory Malmo General Hospital Malmo Sweden

ABSTRACT Defective fibrinolytic activity is often a contributory factor in deep venous thrombosis A family with a high incidence of venous thrombosis in association with such a defect is presented Of 13 family members who had had thrombosis 12 showed a defective *capacity to release fibrinolytic activity* from vessel wall after venous occlusion and/or infusion of DDAVP, a vasopressin derivate The fibrinolytic activator activity of the vessel wall was normal in all cases This seems to be the first family in which there is evidence of an inherited abnormal fibrinolytic activity

Pathogenetic factors in the development of deep venous thrombosis (DVT) are disorders of components of the circulating blood and of the vessel wall Thus a selective increase of certain coagulation factors (6 24) as well as of inhibitors of plasminogen activation (2 18 22) has been found in patients with a severe thromboembolic disease An abnormally low level of antithrombin III is sometimes associated with an increased tendency to develop DVT (3 5 7 14 15 16 26) As for vessel wall changes predisposing to DVT a decreased fibrinolytic activity in the endothelial cells alone or combined with an impaired release of such activity has been found in about 70% of all patients with recurrent thrombotic disease without any other known predisposing components (13) In some patients the connective tissue has an increased tendency to aggregate platelets (10)

This paper concerns the familial occurrence of thromboembolic disease associated with an impaired release capacity of fibrinolytic activity from the vessel wall

PATIENTS AND METHODS

Seventeen members of the family were studied All the tests were performed in a non acute stage at least 4 weeks after an episode of DVT

Laboratory methods

The following determinations were made *platelet count platelet adhesiveness prothrombin+proconvertin+factor X factor V factor VIII activity factor VIII related antigen fibrinogen plasminogen antithrombin III α_2-macroglobulin fibrin/fibrinogen degradation products (FDP) inhibitors of the plasminogen activation euglobulin clot lysis time and fibrinolytic activity of resuspended euglobulin precipitate* of plasma on unheated fibrin plates The procedures have been described elsewhere (4 8 11 12 19 20 21 27) *Fibrinolytic response to venous occlusion* of the arms was assessed according to Robertson et al (25) The fibrinolytic activity of resuspended euglobulin precipitate on unheated fibrin plates was determined after 20 min of venous occlusion Human fibrinogen was used for the fibrin plates The control group consisted of 52 apparently healthy volunteers All persons investigated were examined on 2 consecutive days and afterwards at monthly intervals 95% confidence interval 310–380 mm² *Desamino D arginine vasopressin (DDAVP) test* was carried out as follows DDAVP 8 μg in 100 ml saline was administered as a 30-minute infusion Blood samples were drawn before immediately after and 30 min after the infusion Determinations were made of the fibrinolytic activity of resuspended euglobulin precipitates on fibrin plates The control group consisted of 20 apparently healthy young males Normal range before DDAVP infusion 40–160 mm² immediately after the infusion 140–320 mm² 30 min after the infusion 110–265 mm² (1)

Fibrinolytic activity in walls of superficial veins A segment of a hand vein excised under local anaesthesia was examined with Pandolfi s modification of Todd s fibrinolysis autography technique by Pandolfi et al (23) The activity was expressed in arbitrary units The median value at our laboratory in 70 biopsy specimens of hand veins from healthy volunteers is 7 5 arbitrary units (range 6–10)

Family study

Fig 1 shows the pedigree and Table I the results of investigations of 17 family members 13 with a history of DVT

The first member examined in the family was a boy (IV 26) born in 1959 In 1974 at the age of 14 he was admitted to hospital [illegible]

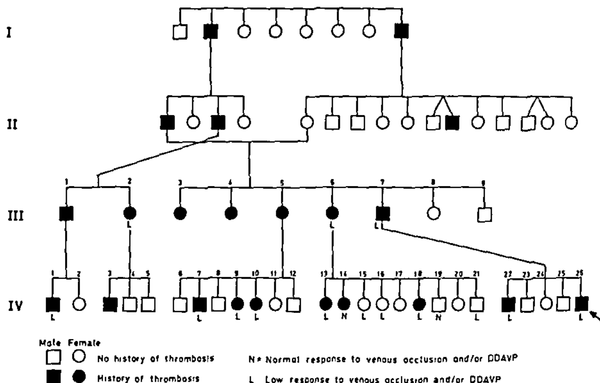


Fig 1 Pedigree of the family (↗ = propositus)

lebography revealed thrombosis occluding the deep venous system including the left iliac vein Chest X ray pulmonary scintigram and angiography showed signs of bilateral embolism Treatment was started with streptokinase and followed by heparin and dicoumarol The boy recovered and was sent home with instructions to take dicoumarol regularly So far he has not shown any signs of a recurrence

Laboratory studies revealed that the release of fibrinolytic activity from the vessel wall was deficient The response to venous occlusion was abnormally weak as was that to stimulation with DDAVP The number and function of the platelets the coagulation factors plasminogen concentration antithrombin III inhibitors of plasminogen activators and α_2 macroglobulin were all normal Many of the patient s relatives had thrombo embolism The pedigree included a marriage between cousins

The propositus elder brother (IV 22) born in 1943 had an attack of DVT of unknown cause in 1971 Since then he has regularly taken dicoumarol and has not had any re currence It was found that he too had a defective release mechanism of fibrinolytic activity

Among their 21 cousins and second cousins in genera tion IV 8 had had episodes of DVT—6 on two or more occasions IV 3 had his first episode at 11 years of age and has since had DVT of one arm Of 12 of the cousins and second cousins investigated 9 proved to have an isolated defective fibrinolytic release mechanism after venous oc clusion (6 with a history of DVT) and one (IV 3 with first DVT at 11 years) had such a defect at the first investiga tion as well as slightly elevated FDP and factor VIII activi ty Later examinations revealed nothing remarkable

The propositus father (III 7) had had recurrent DVT in his twenties and thirties most probably complicated by pulmonary embolism Examinations revealed a defective release of fibrinolytic activity but otherwise nothing re markable Moreover 4 of his 6 siblings had had DVT Only one of them was examined an older sister with an earlier history of DVT All her laboratory results were normal except for a poor response of the fibrinolytic activity to DDAVP Also 2 of the cousins in the same generation (III) had had DVT and one of them was avail able for examination She responded poorly to venous oc clusion but normally to DDAVP

Thus of the 17 family members investigated and in cluded in Table I 13 had had DVT Of these 13 patients the response of the fibrinolytic activity to venous occlu sion and/or DDAVP was weak in 12 In 7 of them the response was poor in both tests in 3 it was poor only after venous occlusion and in one only in the DDAVP test The remaining 2 patients were examined with only one of the tests In one tested with venous occlusion the response was poor and in the other the response to DDAVP was normal

Of the 4 persons examined who had had no episode of DVT in their history one responded normally to venous occlusion (DDAVP was not performed) one normally to DDAVP but poorly to venous occlusion one poorly to both tests and one poorly only to venous occlusion

Table I *The fibrinolytic activity of resuspended euglobulin precipitate on fibrin plates after venous occlusion (normal >310 mm²) and immediately after DDAVP infusion (normal >140 mm²) and the fibrinolytic activity in biopsy specimens in superficial veins (normal >6 arbitrary units)*

Generation no	Venous occlusion mm² Day 1	Day 2	DDAVP (mm²)	Biopsy (arbitrary units)	No of DVT
III 2	500	286	131	–	>2
III 6	403	343	75	7 0	>2
III 7	392	127	113	-	>2
IV 1	396	59	–	8 75	>2
IV 3	399	84	64	–	>2
	383			8 25	
	493	282			
IV 7	344	270	136	7	2
	415				
IV 9	201	126	90	7 0	1
IV 10	390	102	182	9 5	1 (Superficial thrombophlebitis)
IV 13	320	115	110	9 0	>2
IV 14	428	359	–	10 25	>2
IV 15	424	237	–	9 75	0
IV 16	245	240	81	–	0
IV 18	401	254	165	9 0	2
IV 19	328	331	–	8 0	0
IV 21	259	277	214	9 25	0
IV 22	323	109	69	6 5	1
IV 26	106	88	38	6 5	1

DISCUSSION

In about 70% of their patients with spontaneous recurrent DVT Isacson and Nilsson (13) found the fibrinolytic activity in the vessel wall and/or the capacity to release such activity into the circulating blood to be decreased after venous occlusion Many of the members of the family presented in this paper had had DVT We investigated 13 members with and 4 without DVT in their histories Of the 13 patients found to have had DVT 12 repeatedly showed a defective capacity to release fibrinolytic activity from the vessel wall after venous occlusion and/or DDAVP infusion The fibrinolytic activator activity of the vessel wall was normal in all

Only 2 of the 11 patients with decreased fibrinolytic activity after venous occlusion had low values on both days they were investigated The remaining 9 showed a normal fibrinolytic activity at the first examination but unlike normals a significantly low value at the second This indicates an abnormally early exhaustion of the fibrinolytic release capacity The findings also stress how important it is to assess the fibrinolytic capacity on 2 consecutive days One of the family members with repeated attacks of DVT in his history showed a defective release capacity at the first examination as well as mild reactive signs with elevated factor VIII activity and FDP On later occasions the laboratory values were normal This might indicate that the release capacity may vary from time to time perhaps in association with various forms of reactive processes IV 14 who had had DVT but showed normal fibrinolytic activity after venous occlusion (DDAVP test was not performed) had her first attack of DVT shortly after delivery Since then she had had several relapses of DVT of unknown cause

Combined ethyloestrenol and phenformin can normalize the fibrinolytic capacity of a defective vessel wall Nilsson et al (17) found that normalization of the capacity tended to reduce the frequency of the thromboembolic episodes in 75 patients treated for periods of 3–48 months A phenformin like substance is being tried as a single drug (9) In the family presented in this paper 7 members all with the special release defect described are receiving treatment with this drug It is still too early to evaluate the effect

It appears that this is the first family with evi

dence of heredity of an increased frequency of DVT in association with a defective release capacity of fibrinolytic activity from the vessel wall We therefore feel it important to examine all young persons who have had one or more attacks of DVT of unknown cause and in selected cases to extend the investigations to include relatives

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Analysis of Extramedullary Erythropoiesis in the Spleen by a Semiquantitative Method Using Indium-111

R A Rojer N H Mulder H O Nieweg M R C P Edin
H Beekhuis D A Piers and M G Woldring

From the Division of Haematology Department of Medicine and the Department of Nuclear Medicine University of Groningen Groningen the Netherlands

ABSTRACT A semiquantitative method for evaluating the splenic uptake of ^{111}In is described With this method the uptake of indium in the spleen was significantly higher in seven patients with extramedullary erythropoiesis (EME) than in a control group of seven patients with comparable degrees of splenomegaly but without clinical and/or histological signs of EME The discrimination between these groups could be further improved by also taking the degree of splenomegaly into account It is concluded that the described technique is a valuable non invasive aid for establishing the presence of EME in the spleen

A convenient non invasive technique for establishing the presence of extramedullary erythropoiesis (EME) in the spleen would be a useful tool in diagnosis and treatment (7) of myeloproliferative diseases

Labeled iron may be used for this purpose Ferrokinetic measurements with ^{59}Fe (7) are time consuming and hardly practical when serial studies are required These disadvantages do not hold for ^{52}Fe (21) However ^{52}Fe is not available in most clinical centres because of its short half life (8 2 hours)

It has been reported that transferrin bound indium is transported into erythroid precursors (11) Once cell associated it is bound to haem (1) Being similar to iron in these respects it has been suggested that ^{111}In might be used for localizing erythropoietic activity (6 20) The half life of ^{111}In is conveniently long (2 81 days) to permit distribution to centres remote from the site of production Furthermore ^{111}In has suitable detection characteristics

Subsequently however some doubt has been raised about the usefulness of ^{111}In McIntyre et al (12) and Merrick et al (14) demonstrated that marked differences exist between the metabolic behaviour of indium and iron McNeil et al (13) reported that the spleen was clearly visible on the indium scintigram of a patient in whom no evidence of EME was found when the spleen was examined histologically

In the present study we have tried to evaluate the usefulness of ^{111}In for establishing the presence of EME in the spleen by applying a semiquantitative scanning method The essential feature of this method was an attempt to correct the measured activity of ^{111}In over the spleen for the non specific accumulation of ^{111}In in the circulation and for the size of the spleen We therefore compared the corrected quantitated uptake in the enlarged spleens of patients with myelofibrosis with the uptake in the enlarged spleens of patients in whom clinical signs of EME were absent

PATIENTS AND METHODS

Fourteen patients were studied at the Department of Nuclear Medicine Pertinent data concerning age sex diagnosis length of the spleen blood haemoglobin (Hb) concentration and the presence of peripheral leuko-erythroblastosis are provided in Table I Bone marrow biopsies obtained with the Jamshidi needle did not show evidence of myelofibrosis except in patients 1–6 Biopsy specimens were examined by Prof A Arends M D and R Eibergen M D Department of Pathology

Myelofibrosis (patients 1–6) was diagnosed when a deposition of fibrous tissue (collagen and/or reticulin) (10) and clusters of atypical megakaryocytes or megakaryoblasts (24) were found in the bone marrow biopsy specimen Leuko-erythroblastosis and teardrop erythrocytes in May-Grunwald-Giemsa stained smears of peripheral blood were always present Peripheral leuko-erythroblastosis (18) is considered to be the cardinal clinical sign of EME (2) Additional findings (25) included bizarre or giant thrombocytes in the peripheral blood smear evolution from polycythaemia vera and finally evidence of osteosclerosis at X ray examination Splenomegaly was

Table I *Personal data and scanning results*

PLE=presence of peripheral leuko-erythroblastosis in May-Grunwald Giemsa stained smears MSD=maximum splenic diameter as measured on the posterior image of the ^{99m}Tc colloid scintigram S NS=spleen non spleen ratio of ^{111}In activity Q=quotient between S NS and the maximum splenic diameter

Pat no	Age (y)	Sex	Hb (g/100 ml)	PLE	MSD (dm)	S NS	Q	Diagnosis
1	55	♀	14 1	+	2 2	3 8	1 7	Myelofibrosis
2	75	♀	12 2	+	1 5	2 9	1 9	Myelofibrosis
3	76	♂	14 8	+	2 2	4 4	2 0	Myelofibrosis
4	55	♂	6 2	+	2 5	5 3	2 1	Myelofibrosis
5	60	♀	11 9	+	2 5	6 3	2 5	Myelofibrosis
6	79	♂	8 2	+	1 2	3 3	2 7	Myelofibrosis
7	30	♂	12 9	+	2 3	3 8	1 6	Chronic myeloid leukaemia
8	70	♂	9 6	−	2 2	1 5	0 7	*Smouldering monocytic leukaemia*
9	71	♂	10 0	−	2 0	1 7	0 8	Chronic lymphatic leukaemia
10	68	♂	10 1	−	2 8	2 6	0 9	Chronic lymphatic leukaemia
11	63	♂	10 3	−	2 6	1 3	0 5	Hairy cell leukaemia
12	47	♀	11 0	−	2 4	2 7	1 1	Alcoholic liver cirrhosis
13	69	♂	11 3	−	2 7	1 8	0 6	Brill Symmers lymphoma
14	37	♂	15 2	−	1 2	1 2	1 0	Normal volunteer

always found at some stage of the disease In patient 6 the spleen became palpable shortly after the scanning studies

Chronic myeloid leukaemia was diagnosed in patient 7 because of extreme leukocytosis with immature myeloid cells in the peripheral blood smear a low score for leukocyte alkaline phosphatase activity and presence of the Ph' chromosome The cytogenetic study was performed by Dr W L Gouw Department of Human Genetics

Smouldering monocytic leukaemia was diagnosed in patient 8 because of persistent peripheral pancytopenia high level of serum muramidase and an increase of abnormal monocytoid cells in the bone marrow

Chronic lymphatic leukaemia (patients 9-10) was diagnosed when a monoclonal B lymphocyte population was present (tested by membrane bound immunoglobulin fluorescence) In patient 9 a high percentage of cells showed spontaneous formation of rosettes with mouse red cells No fibrosis was found in the bone marrow biopsies which were consistent with the diagnosis of chronic lymphatic leukaemia

Hairy cell leukemia (patient 11) was based on the occurrence of splenomegaly positivity of a significant percentage of peripheral blood cells for acid phosphatase in the presence of tartrate and the observation of irregular cytoplasmic villi in these cells in both May-Grunwald Giemsa stained slides and electron microscopic preparations (5 15)

Portal hypertension (patient 12) caused by alcoholic cirrhosis of the liver was established by liver vein catheterization percutaneous needle biopsy of the liver and peritoneoscopy Pancytopenia was considered to be due to splenic pooling of blood cells as the stimulation test with epinephrine and the bone marrow were normal

Remaining patients (patients 13-14) Patient 13 was scanned prior to diagnostic splenectomy Patient 14 is a presumably healthy subject who gave his informed consent to the scintigraphic examinations

Scintigraphy with ^{99m}Tc colloid and ^{111}In chloride After obtaining a liver-spleen scan (3 mCi of ^{99m}Tc colloid gamma camera) and measuring the maximum splenic diameter on the posterior view of the patient a dose of 2.5 mCi of carrier free ^{111}In chloride (pH 1 1) was injected i v (Duphar Petten The Netherlands) the radiopharmaceutical was not pretreated in vitro (13) The scintigram was performed 48 hours after the injection of ^{111}In chloride using a moving bed gamma camera system Photons of 246 keV were measured in a 25% window

Spleen non spleen ratio As soon as the total body scintigraphy was completed a digital computer was used to registrate the distribution of activity in equal size regions of interest lying within and outside the splenic area on the posterior view of the patient Data were collected during 3 min Following background subtraction the mean splenic activity was calculated from the number of counts in three different regions of the spleen Care was taken to ensure that regions of interest were not lying close to the splenic margin Similarly following background subtraction the mean non-spleen uptake was calculated from the number of "counts" in symmetrical regions over the left and right lungs which did not display activity of ^{111}In on the monitor Subsequently the spleen non spleen ratio was calculated by dividing the mean splenic activity by the mean non spleen uptake The standard deviation of these quotients did not exceed 6%

Ferrokinetic studies Studies with ^{59}Fe were performed shortly after the examinations with ^{111}In in patient 7 The procedure used has been described in detail by Finch et al (7) ^{59}Fe (5 μCi specific activity 3-20 mCi/mg) was preincubated as ferrous citrate with the patient s plasma during 30 min and injected i v External counting over organs (including the spleen) was performed daily for nine days with a single probe collimated NaI detector

Spleen biopsies Splenectomy was performed in patients 11 and 13 a few weeks after the scanning studies with indium Their spleens were examined histologically

Statistics Statistical analysis of the differences in spleen non spleen ratios between the group with myelofibrosis and/or peripheral leuko-erythroblastosis and the



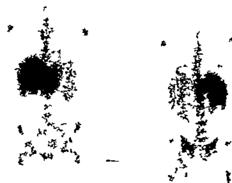


Fig 1 Uptake of ¹¹¹In chloride as visualized on the scan of patient 1 with myelofibrosis Anterior view to the left posterior to the right The activity over the spleen is distinctly higher than in the body background

Fig 2 Scintigraphy with ¹¹¹In chloride in patient 12 with splenomegaly due to portal hypertension in whom clinical signs of EME were absent Anterior view to the left posterior to the right The contrast between splenic area and body background is not as great as on the scintigram in Fig 1 Nevertheless the spleen is readily visible

group without clinical signs of EME was estimated by Wilcoxon s rank sum test

RESULTS

Scintigrams of ¹¹¹In activity The spleen was visualized on the scintigrams of all 14 patients In patients with myelofibrosis the image of the spleen was strikingly clear (Fig 1) However a considerable degree of splenic visibility was also found in some patients in whom clinical signs of EME were absent (Fig 2)

Spleen non spleen ratio It may be seen (Table I Fig 3) that the spleen non spleen ratio was higher than 2 9 in all patients who showed peripheral leuko-erythroblastosis When peripheral leuko erythroblastosis was absent the ratio was below 2 7 Overlap did not occur between the former group (showing clinical signs of EME) and the latter in which splenomegaly was due to other causes The difference between these groups is significant ($p<0\ 005$) It is clear from Fig 3 that the two groups are comparable to each other as far as the degree of splenomegaly in the investigated patients is concerned

Correlation with splenic size The right hand column in Fig 4 shows the result of dividing the spleen non spleen ratio by the maximum splenic diameter (as measured on the posterior view of the ⁹⁹ᵐTc colloid scintigram) The rationale of this procedure is discussed later It is evident from Fig 4 that the discrimination of groups is improved by relating the spleen non spleen ratio to the splenic size

Ferrokinetic measurements (Fig 5) indicated that EME was present in patient 7 with chronic myeloid leukaemia the uptake in the spleen was high initially and started to decrease after the second day (22) A high spleen non spleen ratio of ¹¹¹In uptake had been found in this patient

Spleen histology Examination of the spleen revealed that splenomegaly was caused by nodular centroblastic centrocytic lymphoma (Brill Symmers type) in patient 13 Histological findings in the spleen of patient 11 were compatible with the diagnosis of hairy cell leukaemia EME was not found in the spleen of either of these patients The spleen non spleen ratio had been low in both of them

DISCUSSION

Formal proof of EME was not obtained in the present study since the spleens of our patients with myelofibrosis were not examined histologically In vasiv [illegible] required in order to establish the pr[illegible] histological

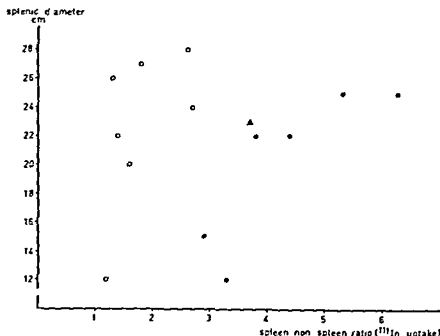


Fig 3 Vertical axis maximum splenic diameter (measured on the posterior image of the ^{99m}Tc colloid scan) Horizontal axis the spleen non spleen ratio of ^{111}In activity ● = Patients 1–6 with myelofibrosis ▲ = patient 7 with chronic myeloid leukaemia ○ = patients 8–14 without clinical and or histological evidence of EME

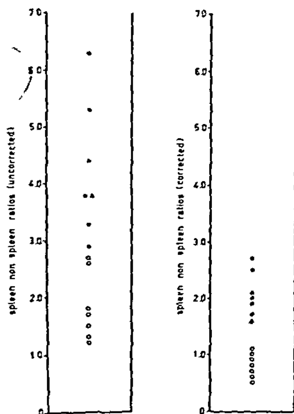


Fig 4 To the left spleen non-spleen ratios of ^{111}In activity To the right, result of dividing the spleen non spleen ratio by the maximum splenic diameter (dm) Symbols as in Fig. 3

However as patients with myelofibrosis frequently show bleeding tendencies caused by both quantitative and qualitative platelet defects (4) invasive procedures are not without risk

There is a consensus in the literature about the presence of EME in the enlarged spleens of patients with established myelofibrosis (27) The spleen non-spleen ratio of indium uptake was high in all our six patients with myelofibrosis It was also high in patient 7 with chronic myeloid leukaemia in whom the presence of EME in the spleen was indirectly confirmed by ferrokinetic studies

Concerning the possibility that EME might have been present in the other conditions we studied (patients 8–14) EME may occur in the spleen of patients with chronic lymphatic leukaemia (23) if this disease is associated with myelofibrosis (secondary myelofibrosis) An association with myelofibrosis was ruled out in our control group (pats 8–14) To our knowledge EME has not been described in smouldering monocytic leukaemia (patient 8) The presence of EME was ruled out in patients 11 and 13 by histological examination of the spleen So there is no reason to assume that EME could have been present in our control group The spleen non spleen ratio of indium uptake was significantly lower in this group than in the group with myelofibrosis

The use of indium as a scanning agent for erythropoietic activity must be approached with cau

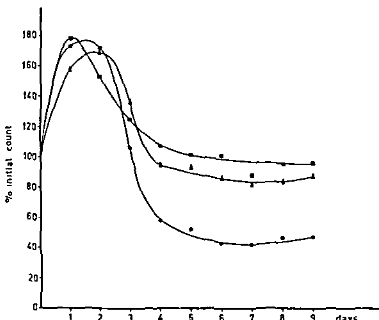


Fig 5 Surface counting pattern in patient 7 following injection of ^{59}Fe ▲—*Spleen* ■=*liver* ●= sacrum

tion as the metabolic behaviour of indium appears to differ from that of iron Compared with iron the binding rate of indium to transferrin is slower (14) It accumulates in the bone marrow to a lesser extent and the percentage of its incorporation in circulating red cells is much lower (12) Iron is incorporated into haem in its bivalent state and indium only exists in the trivalent state (14) so the binding of indium to haem (1) is probably less efficient This might well be the explanation for some of the above mentioned quantitative differences between these elements

In patients without EME the spleen is visible on the ^{111}In scintigram (Fig 2) (13) but not on the ^{52}Fe scan (22) However the present study indicates that ^{111}In may still be used to establish the presence of EME in the spleen provided that the splenic uptake is evaluated in a semiquantitative manner (Fig 3)

The reason for the non specific splenic uptake of ^{111}In when EME is absent is unknown It has been suggested (13) that ^{111}In chloride may be phagocytized by cells of the reticuloendothelial system However 48 hours after injection a substantial amount of ^{111}In is known to be present in the circulation (12) Pooling of blood in enlarged spleens may therefore contribute to the non specific uptake of ^{111}In in this organ It may be remembered that ^{113m}In was originally used as a scanning agent for the blood pool (21)

It is clear from Fig 3 that the measured activity of ^{111}In is related to the size of the spleen in the group without EME for the highest spleen non spleen ratios were found in the patients who had the largest spleens To some extent a relationship between splenic size and indium uptake is also present in the group with EME patients with EME who showed the lowest ratios had the smallest spleens It does not seem likely that the relation between uptake and splenomegaly is entirely due to the degree of EME in patients with myelofibrosis Histological studies by Soderstrom et al (19) indicated that there is no constant relationship between the degree of EME in the spleen and its size Pettit (16) also reported a poor correlation between splenic uptake of ^{52}Fe and splenic size It is obvious that causes of non specific uptake of ^{111}In which hold for the group without EME are also valid for the group with EME This would explain at least part of the relation between splenic uptake of ^{111}In and splenic size in patients with EME

As the uptake of indium is related to the size of the spleen in both groups the degree of splenomegaly must be taken into account when the spleen non spleen ratio is evaluated Regions of interest were projected on the posterior image of the patient Hence it follows that the splenic activity per region will be influenced by the dorso-ventral dimensions of the spleen In order to correct for the degree of splenomegaly the spleen non

spleen ratio would have to be divided by the dorso-ventral diameter of the spleen

In conditions affecting the spleen diffusely the mutual proportions of splenic measures will remain grossly the same when the spleen grows larger (9) Since the spleen was diffusely affected in the conditions we studied an approximately linear correlation may be assumed in our series between the maximum splenic diameter (measured on the posterior view of the ^{99m}Tc colloid scan) and the dorsoventral diameter So we tried to correct the ^{111}In activity for the degree of splenomegaly by dividing the spleen non spleen ratio by the maximum splenic diameter (Fig 4) It may be seen that the discrimination of groups which was achieved by measuring the spleen non spleen ratio of ^{111}In activity (Fig 4 to the left) is improved by correcting this ratio for splenic size (Fig 4 to the right)

Adverse reactions were not observed in our patients nor in 100 patients who were investigated with ^{111}In by Weinstein and Miale (26) The radiation dose is low (11) Because of its safety and ideal physical characteristics (17) ^{111}In is considered suitable for clinical use It may be concluded from our study that a semiquantitative scanning technique using ^{111}In can be a valuable non invasive establish the presence of EME in the spleen

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Conversion of the Electrophoretic Pattern of Type IV Hyperlipidaemia to Type III by Intravenous Heparin

A G Olsson L A Carlson and K Carlson

From King Gustaf V Research Institute and the Department of Internal Medicine Karolinska Hospital Stockholm Sweden

ABSTRACT *Heparin was given i v to subjects with* **type IV hyperlipoproteinaemia who had only ordinary pre β lipoproteins and no late pre β lipoproteins (LP β) in their very low density lipoproteins (VLDL, d<1 006) upon agarose gel lipoprotein electrophoresis Within 15 min the electrophoretic pattern of VLDL had changed completely The normal pre β lipoproteins had disappeared and a discrete LP β lipoprotein had appeared This new electrophoretic pattern induced 15 min after heparin, is similar to that diagnostic of type III hyperlipoproteinaemia It is suggested that the LP β lipoproteins represent an end stage in the catabolism of VLDL**

Type IV hyperlipoproteinaemia (HLP) is characterized by increased concentration of very low density lipoproteins (VLDL d<1 006) with pre β mobility on e g agarose gel lipoprotein electrophoresis of whole serum or of VLDL (1 9) (Fig 1 left) VLDL triglyceride (TG) concentrations are increased and the ratio cholesterol/TG of this lipoprotein is usually about 0 5 (mmol/mmol)

One diagnostic criterion of type III HLP is a lipoprotein band of β mobility on paper electrophoresis present in VLDL (β VLDL) (1 9) (Fig 1 right) which sometimes results in a broad β' band on lipoprotein electrophoresis of whole serum In type III HLP VLDL TG levels are increased and the VLDL cholesterol/TG ratio is elevated often more than 0 8 Type III and type IV HLPs are regarded as different metabolic disorders and have a different clinical picture

The β VLDL were originally believed to represent a unique heritable lipoprotein abnormality The use of Noble s more sensitive lipoprotein electrophoresis on agarose gel (17) instead of paper electrophoresis revealed that in agarose gel the mobility of β VLDL was slightly faster than that of β (3) Furthermore with the agarose gel electrophoresis *discrete* bands with mobility between pre β and β as well as very close to β so called late pre β VLDL [1] LP β lipoproteins are found in the VLDL fraction (d<1 006) in addition to the normal pre β band in both normo- and hypertriglyceridaemic sera in about 30% (3 4)

It has been suggested that the β VLDL of type III HLP (14) and the LP β (3) represent so-called remnants (20) or intermediary particles which are formed during the catabolism of VLDL after removal of a large part of its TGs by the action of lipoprotein lipase To test this hypothesis we increased the catabolism of circulating VLDL by lipoprotein lipase by releasing this enzyme into blood with heparin We show that when heparin is given i v to subjects with type IV HLP *without* LP β lipoproteins there is a rapid generation of LP β lipoproteins and that the electrophoretic lipoprotein pattern may be changed completely into a type III HLP pattern

MATERIAL AND METHODS

Subjects with type IV HLP who completely or almost completely lacked LP β lipoproteins in VLDL in agarose gel electrophoresis were studied in the morning after an

[1] The terminology *late* pre β (LP β) was pref pre β in order to avoid confusion with the nology SPB for *sinking* pre β !

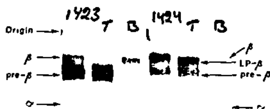


Fig 1 Lipoprotein agarose gel electrophoresis of a type IV (1423) and a type III (1424) HLP (fasting samples) Three runs on each serum from left to right whole serum the top (T d<1 006) and bottom (B d>1 006) fractions after ultracentrifugation at d=1 006 The patient with type III HLP had a classical clinical picture with xanthoma tuberosum on the elbows and xanthoma planum in the creases of the hands Both HLPs had raised VLDL but the electrophoresis of the top fraction shows that in 1423 there were only lipoproteins with pre β mobility while in 1424 there was both pre β lipoproteins and diagnostic for type III HLP so-called β VLDL or LP β as we call them as they always run somewhat faster than the β lipoproteins in agarose gel

overnight fast Blood was drawn in the fasting state and 15 and 60 min after an i v injection of heparin (100 U/kg) collected into EDTA tubes (1 mg/ml of blood) with added merthiolate (Thimerozal 0 1 mg/ml) and immediately placed on ice in order to minimize in vitro lipolysis All urther sample handling was performed at 0–+4 C Blood centrifuged as soon as possible at 2 000 rpm for 10

re ml of plasma from each sample was transferred to a trifuge tube and saline d=1 006 was layered on top of rum to fill the tubes The tubes were then centrifuged in a 75 Ti rotor in a Beckman Model L5-75 ultracentrifuge for 6 5 hours at +4 C at 75 000 rpm VLDL was recovered by slicing the tubes

Agarose gel electrophoresis was performed according to Noble (17) with slight modifications (2) Agarose was obtained from Miles Seravac The conditions were Veronal buffer pH 8 6 μ=0 05 albumin (Pentax bovine albumin fraction V powder) concentration 0 25% (only in the gel plate and not in the buffer) 16 V/cm for 50 min cooling with tap water The strips were stained with Sudan Black

The cholesterol and TG contents in VLDL were determined in two studies by methods described earlier (2)

RESULTS

A typical example is shown in Fig 2 Before heparin was given the subject had an increased pre β band characteristic of type IV HLP with no or only faint colouring at the site of the LP β bands Fifteen minutes after heparin the pre β band had completely disappeared Instead a LP β band of close to β mobility β VLDL had developed Such a band has been considered diagnostic for type III HLP (Fig 1 right) Sixty minutes after heparin the LP β band was still present in the top fraction but in addition a faint pre β band had reappeared The LP β lipoprotein band produced by heparin was sharp and discrete both at 15 and at 60 min after heparin indicating the presence of a reasonably stable distinct lipoprotein class

The increase in the ratio cholesterol/TG in VLDL after heparin further supports the view that heparin virtually had changed the lipoprotein abnormality from type IV to type III HLP (Table I)

DISCUSSION

The new observation reported here is the generation of a discrete LP β lipoprotein 15 min after heparin which turned the electrophoretic lipoprotein pattern of type IV into that considered diagnostic of type III HLP That lipoprotein lipase in vivo and in vitro has pronounced acute effects on serum lipids and lipoproteins has been well known for a long time but the formation of a discrete LP β lipoprotein in response to heparin has not been reported before Graham et al (11) showed in 1951 by analytical ultracentrifugation that heparin reduced the concentration of VLDL with a concomitant increase of denser lipoproteins notably LDL and HDL Further in vivo studies have confirmed these observations (15 16 17) It was reported from this laboratory in 1957 that a heparin induced clearing reaction in vivo as well as in vitro caused a rapid decrease in serum as well as in lipoprotein TG concentration with an increase of monoglycerides (5) Hazzard and Bierman (13) using a combined technique of preparative ultracentrifugation and starch block electrophoresis recently found that heparin

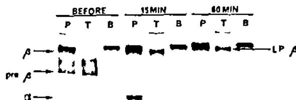


Fig 2 Lipoprotein agarose gel electrophoresis of a type IV HLP before (fasting) 15 and 60 min after i v heparin Three runs on each plasma as in Fig 1 P=whole plasma T=d<1 006 B=d>1 006 Before heparin there is no LP β in the top fraction which only contains VLDL with pre β mobility The appearance of a discrete LP β band 15 and 60 min after heparin is clearly visible in the top (T) fraction

Table I *Concentrations of very low density lipoprotein triglycerides and cholesterol (mmol/l) before and after heparin (100 IU/kg i v)*

	Before heparin	After heparin	
		15	60
Case 1			
TG	4 53	2 67	1 45
Cholesterol	1 89	1 67	1 12
Cholesterol/TG	0 42	0 63	0 77
Case 2			
TG	5 47	3 96	2 09
Cholesterol	2 65	2 30	1 52
Cholesterol/TG	0 48	0 58	0 73

"increased the slower VLDL in one subject with type III and in one with type IV HLP In original work Shore and Shore (21) showed that incubation in vitro of VLDL (S_f 20–400) gave rise to LDL of S_f 11–20 In recent years Eisenberg et al have clarified much of the mechanisms behind the effect of heparin induced lipoprotein lipase on lipoprotein metabolism (7 8 10)

The present concepts can be briefly summarized as follows Lipoprotein lipase acts on the larger very TG rich VLDL by hydrolysing the TG Simultaneously with this removal of TG polar constituents of VLDL such as apolipoprotein C phospholipids and free cholesterol are also removed The process results in the formation of smaller lipoprotein particles remnants (20) or "intermediary density lipoproteins Our present results suggest that the LP β lipoprotein is the end stage—a remnant—of VLDL catabolism a VLDL particle which has lost TGs and apolipoprotein C and become enriched in cholesterol The decrease in electrophoretic mobility from pre β to LP β can probably be ascribed to such losses of apolipoprotein C The discrete band upon electrophoresis indicates a great homogeneity of this lipoprotein class with regard to surface charge properties such as the content of apolipoproteins

Other facts are compatible with this hypothesis that LP β lipoproteins are normal end products in the catabolism of VLDL LP β lipoproteins are not rare Lipoproteins with slower mobility than ordinary pre β are recognized frequently in agarose lipoprotein electrophoresis (3 4 12 17 18) It has to be emphasized however that recognition of LP β lipoproteins requires electrophoresis of *isolated* VLDL as other lipoproteins e g the sinking pre β lipoprotein (SPB or LP(a)) also may appear as discrete bands between pre β and β in electrophoresis of whole serum In a follow up of 609 consecutive lipoprotein analyses of male sera LP β was routinely discovered in 17 26 25 and 28% of normal types II A II B and IV HLP sera respectively (3) The figures for the frequency of LP β are of course dependent on the method which presently is visual inspection The intensity of the band may vary with many factors such as staining In fact we found a frequency of 35% LP β in normal men (4)

Furthermore the presence of LP β is associated with a cholesterol enrichment of VLDL In the study cited above the ratio cholesterol/TG of VLDL in LP β positive compared to LP β negative sera was raised by 37 33 37 and 35% in normals types II A II B and IV HLP respectively (3) In addition to changes in VLDL composition there were changes in LDL composition when LP β was present LDL contained more triglyceride in LP β positive than in LP β negative cases (3) This suggests that when LP β was present LDL contained increased amounts of LDL_1 (d=1 019–1 006) which probably is an intermediary product in the catabolism of VLDL to LDL_2 (d=1 063–1 019)

It remains to be established whether subjects with LP β lipoproteins have an increased rate of formation or a decreased rate of catabolism of LP β In type III HLP both these mechanisms have been suggested as causes for the accumulation of β VLDL (6 19) It also remains to be determined whether the heparin induced LP β lipoproteins and the β VLDL of type III HLP are the same kind of lipoproteins Uterman et al (22) recently suggested that type III HLP is due to a specific apolipoprotein abnormality—a deficiency of apolipoprotein E-III visualized upon isoelectric focussing Further studies are needed to show whether the normally occurring LP β and the LP β induced by heparin display the same pattern

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Myocardial Scintigraphy with ^{99m}Tc-Pyrophosphate in Patients with Unstable Angina Pectoris

Jan Lessem Bengt W Johansson Bertil Nosslin and Jan Thorell

From the Heart Section Department of Medicine and the Department of Nuclear Medicine Malmö General Hospital Malmö Sweden

ABSTRACT *A total of 400 patients,* **aged** *25–82* **years, admitted to the Coronary Care Unit due to clinical suspicion of acute myocardial infarction were examined with 10 mCi ^{99m}Tc labelled to pyrophosphate The examinations were carried out 4–120 hours post onset of symptoms, with a mobile gamma camera Scintigrams were evaluated with regard to presence localization and intensity of an uptake Among 249 patients with a verified acute myocardial infarction, uptake was found in 237 Sixty two of 85 patients with unstable angina showed a diffuse up take with low intensity Scintigraphy could not be used as a prognostic index of which patients would later develop an infarct However the scintigraphic pattern was useful as an aid in the differential diagnosis between acute myocardial infarction and unstable angina**

One of the major disorders in the western world is ischemic heart disease (25) of which both unstable angina pectoris and acute myocardial infarction are common manifestations Unstable angina pectoris is also called preinfarction syndrome a name indicating that a significant number of these patients later developed an acute myocardial infarction (10 14) The diagnosis of myocardial infarction new or old let alone of unstable angina pectoris may be very difficult as ECG and enzyme response may be equivocal or influenced by other concurrent pathological disorders

Recently new methods for the evaluation of myocardial ischemia have been developed and among these several radionuclide methods The main principles utilized are 1) Positive infarct scintigraphy with agents accumulating in infarcted or necrotic myocardium pyrophosphate tagged with ^{99m}Tc (6 24 29 34) or tetracycline tagged with ^{99m}Tc *(19)* 2) *Negative infarct scintigraphy* essentially measuring myocardial perfusion with potassium-43 (35) or potassium analogues (11 28) the most recent being ^{201}thallium chloride (22 33) 3) Scintigraphic evaluation of heart wall movements (32)

However not until recently with the development of the mobile gamma camera has it become possible to use these methods in the coronary care units involving patients with suspected myocardial infarction

The present investigation reports our results using ^{99m}Tc pyrophosphate in patients discharged with a diagnosis of unstable angina

STUDY POPULATION AND METHODS

Four hundred randomly selected patients 313 males aged 25–71 years and 87 females aged 30–82 years admitted to the Coronary Care Unit due to chest pain dysrhythmias pulmonary edema and a primary suspicion of acute myocardial infarction were investigated

A clinical diagnosis of acute myocardial infarction was based on the presence of at least one of the following criteria 1) Subjective symptoms with appearance of typical QRST changes in the ECG 2) Subjective symptoms in the combination with two elevated enzyme values (S ASAT and/or S LD) 3) A combination of ECG changes and two elevated enzyme values (16 17 18 24) 4) Autopsy diagnosis of acute myocardial infarction

The diagnosis of unstable angina pectoris was based on a combination of 1) Stable angina or angina of recent onset with progressive increase and severity of symptoms 2) Angina at rest with recurrent episodes lasting more than 20 min and poorly relieved by nitrates (10 20) 3) No elevation of serum enzyme levels above normal (10) 4) Transient ST T wave changes during chest pain often consisting of T wave inversion ST T segment elevation or depression which frequently when the pain episode is over (14 20)

Table I *Correlation between clinical diagnosis and positive scans*

Diagnosis	No of positive scans	Intensity grade 1	2	3	No of negative scans
Acute myocardial infarction (n=249)	237	25	98	114	12
Unstable angina pectoris (n=85)	62	54	8	0	23
Other cardiac diseases (n=38)	5	2	3	1	33
Non-cardiac disease (n=28)	1	1	0	0	27
Total	305	82	109	115	95

As a rule patients were monitored two or three days in the Coronary Care Unit with two enzyme values daily and a 12 lead ECG once a day

Based on these criteria 213 patients developed a transmural myocardial infarction and 36 subendocardial infarcts Of the remaining 151 patients 85 had unstable angina pectoris 38 developed other cardiac diseases and 28 were discharged without any signs of cardiac disease

All patients were examined 4–120 hours following onset of symptoms with 10 mCi ^{99m}Tc pyrophosphate (Byk Mallincrodt USA Diagnostics Incorporated USA Solvent KABI-diagnostica) administered i v and scintigrams were recorded 60 and 90 min after injection The patients were examined with a mobile gamma camera (Porta camera IIB and C General Electric USA) in the Coronary Care Unit The Porta camera IIB is a fast scintillation camera and has a gamma detector with a 33 cm sodium iodide crystal and 19 photomultiplier tubes while the Porta camera IIC has 37 photomultiplier tubes and also better resolution A standard low energy parallel hole collimator was used In each scintigram 400 000 counts were collected The pyrophosphate scintigrams were obtained in the anterior posterior and in the left 30 degree anterior oblique position All scintigrams were evaluated with regard to presence localization intensity and size of an uptake The intensity was graded 0–3 where grade 1 represents faint uptake and grade 3 a strong uptake in relation to the background activity

RESULTS

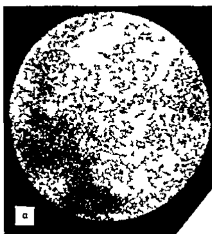
Of the 400 pyrophosphate scintigraphies (Table I) 305 yielded positive and 95 negative findings Of the possitive scans 237 referred to patients with acute myocardial infarction 62 to patients with unstable angina pectoris one to a patient with Prinzmetal angina one to a Chagasic heart three to patients with old infarcts and suspected ventricular aneurysms and one to a female patient with metastatic carcinoma of the breast treated with Doxorubicin

Of the 85 patients who were discharged with a diagnosis of unstable angina 62 (73%) showed a pyrophosphate uptake None of these patients had any enzyme rise above normal values but 73 had an increase in S ASAT within the normal range The pyrophosphate scintigram turned positive already six hours after onset of symptoms in the seven patients examined that early The recorded radionuclide accumulations were spread over a large area of the myocardium which contrasted this group from patients with transmural infarction (Fig





Fig 1 Diffuse pyrophosphate uptake in a patient with unstable angina (*a*) compared with a highly intense uptake in a patient with transmural acute myocardial infarction (*b*) Both scintigrams recorded in LAO 30°



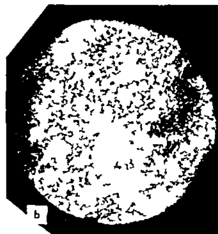


Fig 2 Widespread pyrophosphate uptake in a patient with unstable angina (*a*) compared with very well localized uptake in a patient with anterolateral transmural myocardial infarction (*b*)

1*a* and *b*) The most characteristic feature of an uptake in patients with unstable angina pectoris was the low intensity Eighty seven per cent of the patients exhibiting uptake showed an intensity visually graded 1 This finding was a further distinction between patients with transmural myocardial infarction and those with unstable angina pectoris The patients with transmural myocardial infarction had an intensity graded 2 or 3 (Fig 2*a* and *b*) In this group of patients with unstable angina pectoris none developed permanent ECG changes and there was no correlation between the intensity of an uptake and transient ECG changes Fifteen patients were readmitted after a few weeks and seven had developed an acute myocardial infarction as documented by Q wave evolution in the ECG All were reexamined with another pyrophosphate examination The seven patients who had developed acute infarcts showed well localized grade 2 or 3 intensity pyrophosphate uptakes (Fig 3*a* and *b*) There was no specific scintigraphic pattern in the first recording to indicate that these patients would develop an acute infarct some weeks later

There was no difference in the clinical course of patients with positive or negative pyrophosphate scans in the group of unstable angina In neither were the enzyme changes at any time above normal values



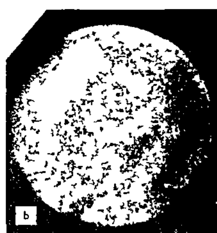


Fig 3 Diffuse uptake in a 54 year-old male who was readmitted to the CCU three weeks later (*a*) and a well localized grade 3 intense pyrophosphate uptake registered in the inferior wall corresponding to newly developed Q waves in leads II III avF and V_5–V (*b*)

DISCUSSION

It has previously been shown that ^{99m}Tc pyrophosphate myocardial scintigraphy is an accurate and sensitive method in diagnosing acute transmural infarction (7 15 23 26 27) Willerson et al (34) could also show that the radionuclide accumulation in patients with acute transmural myocardial infarction was focal intense and well defined This result was later confirmed by others (5 15) Pyrophosphate is thought to have an affinity to hydroxyapatite crystals formed as granules within the mitochondria when the myocardium undergoes necrosis (8 12 31) Recently evidence has been presented that pyrophosphate mainly accumulates in irreversibly damaged myocardial cells (9 30)

In several studies it has been shown that unstable angina pectoris can yield positive pyrophosphate uptakes (1 13 15) Willerson et al (34) first described the characteristic pattern of the uptake later confirmed by Berman et al (5) Most authors have reported 30% positives in unstable angina However Ahmad et al (2) in a small material found that all patients with unstable angina pectoris showed positive pyrophosphate uptakes while Karanuratne et al (21) observed that 85% of pa with unstable angina had positive uptakes In 62 of 85 patients (73%) showed positive a result in concordance with the other reports

The positive scans in patients with unstable angina pectoris may indicate that—compared with ECG and conventional enzyme methods—this is a more sensitive method of registering myocardial necrosis of which there are several different types (3) As pyrophosphate has been shown to mainly accumulate in irreversibly damaged myocardial cells (4 30) it supports the hypothesis that unstable angina is an expression of micronecrosis rather than generalized ischemia No evidence has been presented suggesting that pyrophosphate can accumulate in ischemic myocardial cells

It is important that the scintigraphic pattern of the uptake in patients with unstable angina pectoris differs substantially from that in patients with acute transmural myocardial infarction Therefore myocardial scintigraphy may become an important tool in the differential diagnosis of patients with suspected coronary artery disease and may contribute to more accurate knowledge of the pathophysiology of unstable angina pectoris It does not seem as if pyrophosphate scintigraphy can be used as a prognostic index of which patients with unstable angina will later develop acute myocardial infarction

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Non-Invasive Methods for Evaluating the Importance of Heart Rate and Atrial Activity in Cardiac Pacing

Results of Ballistocardiography and Digital Plethysmography Studies in Six Patients with Heart Block

T Theorell O Edhag and B Fagrell

From the Department of Medicine Karolinska Institutet at Serafimerlasarettet Stockholm Sweden

ABSTRACT Ultralow frequency ballistocardiography (UFB) and digital pulse plethysmography (DPP) were performed in six patients with an external artificial pacemaker system UFB was used mainly to evaluate the force of contraction of the left ventricle (IJ amplitude) and DPP for evaluating relative changes in the peripheral pulse volume Four patients were studied at 40 50, 60 70, 80 90 and 100 beats/min *There was a significant decrease* ($p<0.001$) in IJ and pulse amplitudes when the heart rate increased from 40 to 100 beats/min A positive correlation between relative IJ and pulse amplitude was observed in all cases studied In beat to-beat analysis it was found that the importance of the PR interval for the IJ and pulse amplitudes varied between patients It is concluded that both UFB and DPP may be of value *in clinical practice for evaluating* hemodynamics in patients with slow spontaneous heart rate The methods may be of help in selecting the most effective type of pacemaker for the individual patient

The two most frequently used artificial pacemaker systems nowadays are the ventricular programmed QRS-inhibited or synchronized In some patients with these systems the spontaneous activity is dominated by a slow ventricular rate During periods of such activity the pacing system is mostly programmed to give a fixed pacing rate of 60 or 70 beats/min In the individual patient it may be difficult to know whether the hemodynamic situation is improved *during periods of spontaneously* slow ventricular rate by artificially inducing a higher ventricular rate Furthermore these systems are not suitable for patients in whom coordinated contractions between the atria and the ventricles are essential to avoid *intermittent hemodynamic* disturbances The only way so far to identify patients suitable for such treatment has been to make a heart catheterization and record variations in flow and pressure in relation to the varying PR intervals Non invasive procedures not painful to the patient and easy to repeat would be preferable to invasive methods at least for screening purposes

A combination of two such techniques has been evaluated The first one *ultralow frequency ballistocardiography* provides information mainly about variations in the force of contraction of the left ventricle The second one *digital pulse plethysmography* determines relative variations in the peripheral pulse volume In the present study the possible clinical application and the relationship between the two methods have been evaluated

PATIENTS AND METHODS

The following criteria were used to select suitable patients for the study 1) Externally worn pacemaker (Siemens Elema EM 145) 2) Ability to lie on the ballistocardiographic table 3) Low spontaneous ventricular rate (≤40 beats/min) 4) No acute circulatory distress 5) No technically induced artefacts such as contractions of the diaphragm or involuntary muscle movements

One woman and three men with a mean age of 70 years (range 55–81) were studied Two additional patients (nos 5 and 6) who did not have as slow a ventricular activity as the others but well recognizable P waves were studied with regard to varying PR interval All patients presented in Table I had recently (<2 weeks) received a transvenous pacemaker electrode as well as a subcutaneous indifferent electrode The electrodes were connected with an external pulse generator

Table I *Patient data*

Pat no	Age (y)	Sex	Rhythm	Heart X ray	Heart failure	Etiology/associated disease
1	55	♂	CHB+VT	Enlargement+LV aneurysm	Yes	IHD?
2	81	♀	Sinoatrial block	Slight enlargement	Yes	Thyroid dysfunction
3	74	♂	CHB	Enlarged	Yes	Hypertension
4	68	♂	CHB	Enlarged	Yes	IHD+muscular atrophy
5	82	♂	CHB	Enlarged	Yes	Unknown
6	63	♂	CHB	Not known	No	Recent myocardial infarction

Ballistocardiography

An ultralow frequency ballistocardiography delivered from the Royal Medical Corporation was used The instrument was calibrated 1 cm amplitude corresponded to an acceleration of 2 0 cm/sec² (5)

All recordings were made during normal breathing and with a paper speed of 50 mm/sec At each heart rate the last ten complexes were used for calculation of mean IJ amplitude and mean IJ ratio (amplitude divided by duration) For each subject mean IJ amplitude and IJ ratio were calculated from all recordings The subject s measures at each heart rate were subsequently related to the subject s own mean Thus 1 0 indicates a value equal to the subject s own mean All recordings were performed during steady state at the following heart rates 40 50 60 70 80 90 and 100 beats/min The order of tested heart rates varied in every second case from 60 50 40 80 90 100 to 80 90 100 60 50 40

Table II *Relative ballistocardiographic and plethysmographic measures in relation to heart rate*

IJ amp=relative IJ amplitude IJ ratio=relative amplitude/duration ratio pl=relative plethysmographic amplitude

Heart rate (beats/min)	Pat no	IJ Amp	IJ Ratio	pl
[illegible]	1	1 51	1 36	2 9
	2	1 12	1 11	1 4
	3	1 27	1 07	1 7
	4	1 05	0 92	1 1
50	1	1 20	1 10	2 3
	2	1 06	0 98	1 3
	3	1 04	0 98	1 4
	4	1 02	1 06	1 2
60	1	0 91	0 93	1 9
	2	1 25	1 12	0 9
	3	0 98	0 91	1 0
	4	1 08	0 88	1 2
70	1	0 90	0 84	0 0
	2	0 78	0 83	1 3
	3	0 95	1 09	1 0
	4	1 10	1 03	1 1
80	1	1 04	1 00	0 0
	2	0 94	0 96	0 8
	3	0 92	0 94	0 7
	4	0 90	0 91	0
90	1	0 70	0 75	0
	2	1 01	1 04	0 9
	3	0 92	0 94	0 7
	4	0 88	0 8[illegible]	0 8
100	1	0 75	[illegible]	0 0
	2	0 82	[illegible]	[illegible]
	3	0 95	[illegible]	9
	4	1 01	[illegible]	[illegible]

Plethysmography

The peripheral arterial pulse wave was recorded plethysmographically from the right thumb by a mercury in silastic strain gauge (8) An electrical calibration unit made it possible to calculate digital pulse volume in ml (1 2) In the present study the average amplitude (mm) of the pulse volume of ten consecutive beats was measured in each recording Relative changes at different heart rates in one and the same individual of average plethysmographic amplitude were calculated in a way analogous to the ballistocardiographic data Thus 1 0 indicates a value equal to the subject s own mean

ECG and phonocardiography were recorded simultaneously in all patients studied

RESULTS

Correlation between heart rate amplitudes of IJ and plethysmogram and the amplitude/duration ratio of IJ

Table II shows the individual results at different heart rates

There was a significant decrease in the IJ amplitude when the heart rate increased from 40 to 100
/min The [illegible] l regression line was
al [illegible]) and highly sig
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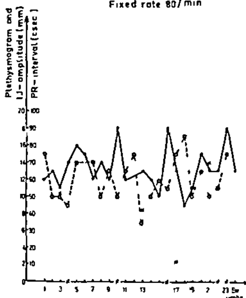


Fig 1 PR intervals in relation to measured parameters in patient 4 ●—●=IJ amplitude ○ ○=pulse plethysmogram × ×=PR interval

Mean relative plethysmographic amplitudes decreased sharply with increasing heart rate The computed ideal regression line was exponential ($y=4\ 71\times 0\ 98^x$) and highly significant ($p<0\ 001$)

A positive correlation between relative IJ amplitude and relative digital pulse amplitude was observed in all cases studied The mean regression ($R=0\ 78\pm 0\ 05$) was highly significantly different from zero ($p<0\ 001$)

Relation between PR interval on the one hand and amplitudes of IJ and pulse volume on the other

Figs 1–3 show beat-to-beat analyses of three patients In patient 6 (Fig 4) the PR intervals varied in a cyclic way increasing gradually from 0 to 60 csec No interference with normal respiratory pattern was seen Relatively large differences between maximal and minimal amplitudes were observed The optimal PR interval for the IJ amplitude (0–10 csec) was shorter than that for the plethysmographic one (15–25 csec)

In patient 4 (Fig 1) the PR intervals also varied cyclically but in reversed order The optimal PR intervals for the IJ and plethysmographic amplitudes were roughly the same as in the first case

In patient 3 (Fig 2) the PR intervals varied slowly while the IJ amplitudes varied sharply with respiration The pulse volume did not seem to be influenced by the respiration In order to determine the relationship between PR interval and IJ amplitude the average IJ amplitude was calculated from five consecutive complexes Extremely long PR

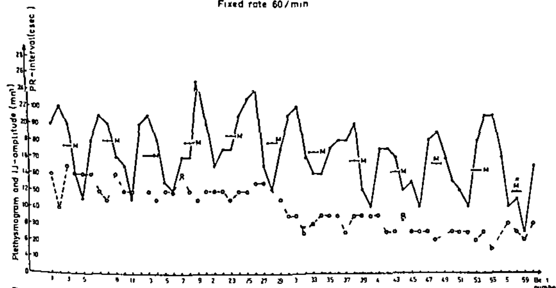


Fig 2 PR intervals in relation to measured parameters in patient 3 –●–=Mean of five consecutive IJ amplitudes Other symbols as in Fig 1

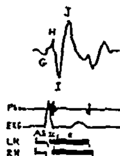


Fig 3 Acceleration ballistocardiography in a normal subject in relation to concomitantly recorded phonocardiography (*Phon*) and electrocardiogram (*EKG*) *LH*=left heart *RH*=right heart *AS*=atrial systole *IC*=isovolumic contraction *E* ejection

intervals were associated with small IJ amplitudes The pulse volume decreased successively with an increase in PR interval

Table III shows the absolute means and standard deviations of amplitudes at different groups of PR intervals in the five patients with visible P waves in ECG The plethysmographic and IJ data are given together with corresponding GH amplitudes The GH wave in the ballistocardiogram corresponds to the summation of recoils produced by 1) venous return to the heart+atrial filling and 2) atrial contraction (5) (Fig 3)

The data show considerable standard deviations for some subjects but uniformly indicate the following (*a*) At marked relative differences between *ethysmographic amplitudes at different PR inter* s ballistocardiographic relative differences are also marked and of the same magnitude (*b*) The optimal PR intervals for the plethysmogram and GH amplitudes are always between 11 and 29 csec *whereas for the IJ amplitude they are shorter* 0–10 csec

Table III *Means and standard deviations of amplitudes (plethysmogram IJ and GH) at different groups of PR intervals in five patients*

Pat no	PR (csec)	Plethysmogram (mm)		IJ (mm)		GH (mm)	
		M	S D	M	S D	M	S D
2	0–10	2 0	–*	16 0	–	3 0	–*
	11–29	4 7	1 2	14 3	2 6	4 8	3 3
	30–	3 5	1 1	11 1	4 0	3 4	2 0
3	0–10	–	–	–	–	–	–
	11–29	11 7	1 8	17 7	3 8	9 1	2 4
	30–	7 4	1 1	14 7	3 8	9 1	2 7
4	0–10	13 7	2 3	17 7	2 4	3 4	3 5
	11 29	15 2	4 7	13 3	2 9	7 7	2 7
	30–	12 8	2 0	14 4	1 4	2 6	1 4
5	0–10	–	–	19 0	5 7	2 8	1 0
	11–29	–	–	18 8	6 5	4 5	1 9
	30–	–	–	13 2	3.3	3 0	2 0
6	0–10	9 1	2 8	13 0	6 4	4 0	2 4
	11–29	11 9	1 2	7 1	3 6	7 0	4 5
	30–	5 9	2 2	3 1	2 0	4 0	1 7

* $n=2$

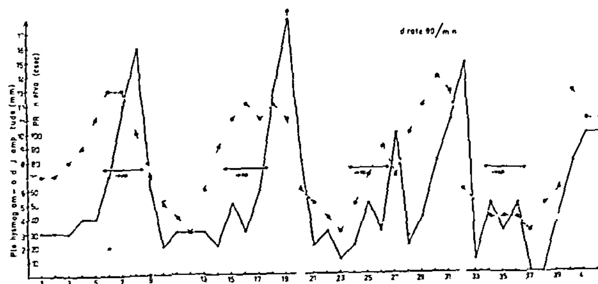


Fig 4 PR intervals in relation to measured parameters in patient 6 Abscissa indicates beat number Symbols as in Fig 1

DISCUSSION

Effects of artificial heart rate variations

Karlof et al (3) measured invasively beat to-beat changes in stroke volume during sudden artificial changes in heart rate and found that the left ventricle adapts immediately changing the stroke volume without a corresponding increase in contractility The data obtained in the present study show that some patients were able to change the IJ and digital plethysmographic amplitudes when the heart rate was changed while others were less able to do so The measures obtained with the two methods used varied simultaneously However normal respiration did sometimes influence the ballistocardiographic data considerably (patient 3) The pulse amplitude on the other hand did not seem to do so

The IJ amplitude of the ultralow frequency ballistocardiogram is strongly correlated with the acceleration of flow in the aorta When other relevant variables are held constant the acceleration of flow is dependent on the stroke volume (5) The findings in the present study indicate that the compensatory increase in stroke volume taking place during an artificial slowing of the heart rate is reflected both in the IJ amplitude and in the digital pulse amplitude

The present observation that the IJ ratio (amplitude/duration) did not change uniformly during the manipulations of heart rate is in accordance with the finding of Karlof et al (3) that contractility did not change immediately after artificial changes in heart rate

The conclusion of this part of the findings is that both ultralow frequency ballistocardiography and digital plethysmography may be used in the evaluation of a patient s ability to change stroke volume during artificial heart rate manipulations

Effects of varying PR intervals

The study of the effects of a varying PR interval on the circulation indicates that for some subjects the coordination between atrial and ventricular contraction was of importance whereas for others it was less so The optimal PR interval for ballistocardiographic IJ amplitude on the one hand was shorter than that for plethysmographic or ballistocardiographic GH amplitude on the other The interpretation of this finding is difficult since the GH complex may consist of two recoil components one caused by venous return to the heart plus atrial filling (4) and the other by atrial contraction (7) The atrial contraction component of the GH complex may be diminished or even reversed when the atria and ventricles contract simultaneously due to ejection of atrial blood in the wrong direction This may help to explain the discrepancy between GH and IJ amplitude variations in relation to varying PR interval An alternative explanation may be that the coincidence of body recoils from the left atrial and ventricular contractions at PR intervals close to zero creates a strong recoil which may have no hemodynamic significance per se Ultralow frequency ballistocardiographic observations have been made during anesthesia on spontaneously varying PR intervals Large variations in IJ amplitude were observed when the P wave was marching into or out of the QRS complex (6)

It should be pointed out particularly for the ballistocardiographic data but also for the plethysmographic that the correlations between stroke volume changes on the one hand and changes in amplitudes on the other may be non linear Thus a three fold increase in plethysmographic amplitude may not correspond to a three fold increase in stroke volume and vice versa Despite this large changes in plethysmographic amplitudes may indicate large changes in stroke volume The correlation between the PR interval and the pulse amplitude may also be used for a rough estimation of the effect of atrial activity on stroke volume If no change in pulse amplitude is seen with varying PR intervals atrial contractions have most probably no major effect on the amount of blood expelled from the heart at each beat On the contrary in our patient 6 (Fig 4) a 2–3 fold increase in pulse amplitude occurred when a ventricular contraction was preceded by a P wave compared to one that was not This showed a positive effect on stroke volume by atrial contractions at an optimal interval to the ventricle contraction

The fact that concordant results were obtained with two widely different non invasive methods gives mutual support to the use of both of them for evaluating central hemodynamics The pulse volume is influenced by several factors such as the parasympathetic and sympathetic nerve activity Despite this when the heart rate is varied artificially plethysmographic data seem to correlate well with changes in central hemodynamics as reflected in the ballistocardiogram

The major limitation of ultralow frequency bal

listocardiography is that subjects who are unable to lie on the table for a period of half an hour cannot be studied Furthermore subjects with pronounced tremor cannot be studied Good digital pulse plethysmograms cannot be recorded in patients with pronounced peripheral vasoconstriction Thus the two methods may be complementary Some subjects could be evaluated with the first method but not with the second and vice versa

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ECG Aberrations, Latent Coronary Heart Disease and Cardiopulmonary Fitness in Various Age Groups of Norwegian Cross-Country Skiers

Håkon Lie and Jan Erikssen

From the Institute of Work Physiology and Medical Department B Rikshospitalet Oslo Norway

ABSTRACT To assess the prevalence of possible latent coronary heart disease (CHD) among physically active men, 149 elite cross-country skiers in three age groups (26–33, 43–50 and 58–64 years) were invited for an examination which included clinical examination, Vitalogram, resting ECG, and a near maximal bicycle test Of the invited men 122 participated i e 81 8% The following findings were made Normal clinical findings in all except 2, low resting heart rate, lung function parameters of about normal mean voltage signs of left ventricular hypertrophy in resting ECG in 61/122, incomplete right bundle branch block in 14/122, codable Q waves (Minnesota Code MC) in 5/87 from the highest age groups, ischaemic exercise ECG changes of MC 4 1 or 4 2 types in 11/87 vs 1/35 in the two oldest vs the youngest age group Physical performance was very high in all age groups, but regular training did not seem to inhibit the normal age dependent decline in physical performance The resting and exercise ECG data in the two oldest age groups did not differ favourably from similar data obtained in sedentary men of the same age from approximately the same geographic area Thus it is possible that regular strenuous exercise and training may not protect against the development of CHD The implications of such a view are briefly discussed

During recent years there have been several cases of sudden death among middle aged men while participating in strenuous cross-country competitions in Norway and it has been argued that screening procedures ought to be elaborated in order to avoid such tragedies

Resting ECG changes of various kinds and ST changes noted during exercise tests are known to carry an increased risk of coronary heart disease (CHD) morbidity and mortality (1 8 9 10 14) in asymptomatic men

The main aims of the present study were to assess the cardiopulmonary fitness and the prevalence of various ECG abnormalities at rest and during exercise in well trained Norwegian cross country skiers of different ages in order to assess the number of cases of potential latent CHD

STUDY POPULATION

A total of 149 long time active highly trained cross country skiers belonging to three preselected age groups (group I 26–33 years group II 43–50 years group III 58–64 years) were invited to participate in the study The provisions for entering the study were that they 1) for several years had participated in long-distance cross country competitions (30–90 km) and 2) in general were among the 1/4 with the highest performance in the various contests

Of the 149 men invited 122 (81 8%) came for the examination 35/48 (72 9%) from group I 48/58 (82 6%) from group II and 39/43 (90 7%) from group III This difference in participation rate among the various age groups is not significant ($p>0\ 10$)

The mean training hours/week in the three groups during summer and winter seasons were (apart from hours of competition) 9 1 and 5 3 in group I 7 6 and 5 0 in group II and 8 1 and 5 0 in group III However training time varied widely particularly during the summer season Only 13 men suggested that they had any training effect in their jobs whereas 96 claimed that they had totally sedentary jobs

METHODS

The examination consisted of 1) case history and 2) a general examination program In addition cholesterol and triglycerides were examined as reported elsewhere (19)

The *case history* included indices of angina pectoris smoking habits a history of CHD in first degree relatives and incidents of extreme exhaustion during training and/or competition

The *examination program* included a complete physical examination a vitalographic assessment of vital capacity (VC) and one second forced expiratory volume (FEV 1 0 sec) and a near maximal bicycle exercise test (2) on an *electrically braked Elema bicycle*

At rest a standard 12 lead ECG was recorded *During exercise* the exercise ECG was monitored as reported previously using chest–head leads 1-2 4–7 (11) All resting and exercise changes were classified according to the Scandinavian Modification of the Minnesota Code (MC) (2) In groups I and II the starting load was ~200 W (1200 kpm/min) in group III ~150 W (900 kpm/min) for 6 min directly followed by 6 min on ~250 W (1500 kpm/min) and ~200 W (1200 kpm/min) respectively Thereafter the men had 2 min rest and the load was then successively increased by ~50 W (300 kpm/min) each 3 min The exercise was continued until exhaustion or until ischaemic or other ECG changes necessitated a premature break. Exhaustion and/or leg fatigue were/was the reason(s) for discontinuing the test in all but 2 cases During exercise ECGs were recorded at 2 4 and 6 min during each of the first two loads and immediately before an increase in load (or at break) on all higher loads

Post exercise ECGs were recorded immediately after breaking the test and at 1 2 3 and 5 min To ensure maximal mechanical efficiency the pedalling rate was kept at 60–70/min (18) Maximal oxygen uptake (V_{O_2} max) (l/min and ml/kg/min) was predicted according to strand and Ryhming (3) using data from load 2 (similar es were obtained by using load 1)

RESULTS

No significant cardiopulmonary abnormalities were revealed during the physical examination except atrial flutter in one man and frequent ventricular extrasystoles in another Five (*all* >40 *years*)—none of whom had any symptoms of CHD—had codable Q wave changes in resting ECG (MC 1 1–1 2– and 1 3 types) (Table I)

The prevalence of ischaemic exercise ECG changes (MC 4 1+4 2) in the various groups was as follows (in parentheses MC 4 1 only)

Group I	2 9/100	(2 9/100)
	n=1/35	*n*=1/35
Group II	8 3/100	(4 2/100)
	n=4/48	*n*=2/48
Group III	17 9/100	(7 7/100)
	n=7/39	*n*=3/39

Only one of the 12 men (44 years old) with a positive exercise ECG (MC 4 1 *or* 4 2) (Table II) had suspect coronary pain (half a year prior to this study) None reported a history of angina pectoris nor had any of the men angina during the exercise test The two men mentioned above (aged 58 and 60 years) with frequent ventricular extrasystoles and atrial flutter respectively had recently sought medical advice because of a recent deterioration of physical performance All the 5 cases of post exercise functional 1st degree sinoatrial block—lasting but a few seconds—were triggered by deep inspiration Incomplete right bundle branch block (RBBB) (MC 7 3) was apparent in 14 (11 5/100) voltage sign of left ventricular hypertrophy (MC

Table I *Resting ECG changes noted in 122 cross country skiers*

	Age group		
	26–33 (*n*=35)	43–50 (*n*=48)	58–64 (*n*=39)
Very probable myocardial infarction[a]	0	0	1
Probable and possible myocardial infarction[b]	0	2	2
Atrial flutter	0	0	1
1st degree AV block	2	0	1
2nd degree AV block	1	0	0
WPW syndrome	1	0	0
Complete RBBB	0	1	0
Incomplete RBBB	5	7	2
Sinus bradycardia (<50/min)	15	21	13
Left ventricular hypertrophy[c]	23	25	13
Left axis deviation (<-30°)	0	0	2

[a] Minnesota Code (MC) 1 1– [b] MC 1 2– or 1 3– [c] MC 3 1 (*a*) R amplitude >33 mm in either of leads $CH_{1\ 6 or 7}$ or (*b*) >20 mm in any of leads I II III or a VF or (*c*) >12 mm in lead a VL (2)

Table II *ECG changes during and/or post exercise in 122 cross-country skiers*

	Age group		
	26–33 (*n*=35)	43–50 (*n*=48)	58–64 (*n*=39)
Ischaemic changes	1 (1)	4 (2)	7 (3)
Junction changes[b]	6	14	12
Frequent ventricular extra systoles (≥5/min)	0	0	1
Infrequent ventricular extra systoles (<5/min)	0	3	4
Nodal rhythm post exercise	2	1	1
1st degree sinoatrial block post exercise	5	0	0

[a] Minnesota Code (MC) 4 1 and 4 2 (figures in parentheses represent MC 4 1 only) [b] MC 4 6 and 4 7 (2)

Table III *Maximal heart rate (MHR) resting heart rate (RHR) resting systolic blood pressure (SBP) maximal exercise blood pressure (MSBP) cholesterol and triglyceride values in 122 cross country skiers*

Age group		RHR (beats/min)	MHR (beats/min)	SBP (mmHg)	MSBP (mmHg)	Cholesterol (mM/l)	Triglycerides (mM/l)
26–33	Mean	51 6	173 7	132 7	201 7	5 87	1 23
	S D	9 5	11 1	10 9	20 8	1 07	0 40
43–50	Mean	51 5	164 7	137 2	207 8	6 75	1 24
	S D	9 4	11 5	11 5	23 3	0 95	0 39
58–64	Mean	55 5	156 8	151 4	215 1*	6 98	1 64
	S D	9 2	11 1	15 6	26 2	0 99	0 65

Figures from the man with atrial flutter are disregarded

3 1) in 61 (50/100) and sinus bradycardia (<50 beats/min) (MC 8 8) *in 49 (40 2/100) (Tables I and II)*

The serum cholesterol level was similar to that found in healthy men of the same age in the Oslo region (20) whereas non fasting triglyceride levels were significantly lower ($p<0\ 001$) (Table III) However it is worth noting that neither the present nor the Oslo study blood samples were taken in fasting state (20) Resting heart rate was particularly low in the present population whereas resting and exercise BPs were in general normal Mean maximal heart rate at break of the exercise tests was 174 165 and 157 in the three age groups respectively These heart rates were recorded at the time of physical exhaustion and/or leg fatigue in all but the 2 men mentioned above (Table III)

Only 8/122 smoked cigarettes and only one was a heavy smoker (≥20 cig /day) None was over weight There was a history of CHD in first degree relatives in 32 men but such a case history was unrelated to any of the findings mentioned above

Lung function tests (Table IV) indicate a VC of about normal mean and an FEV 1 0 sec of above normal mean (6) V_{O_2} *max* was considerably higher than in normal individuals in all groups (3) There was a drop of at least 30% from group I to group III as assessed by this indirect method of estimating V_{O_2} max indicating an age-dependent drop in V_{O_2} max among athletes similar to that in sedentary subjects (4)

DISCUSSION

It has been claimed that exercise may prevent CHD On the other hand serious arrhythmias or coronary insufficiency may theoretically be precipitated in susceptible individuals during strenuous physical activity Opie (22) claims that 18/21 deaths which he observed in active sportsmen were caused by acute heart attacks precipitated by (or immediately following) strenuous exercise Shephard (24) estimated that one heart attack per 2500 gymnasium hours would occur in middle aged American men attending unsupervised gymnasium programs The apparently low fatality rate of 10 per 12 million

Table IV *Maximal oxygen uptake (V_{O_2} max) and spirometric values in 122 cross-country skiers*
VC=vital capacity FEV=forced expiratory volume

Age group		V_{O_2} max (ml/kg/min)	Relative V_{O_2} max [b]	VC (l)	Relative VC	FEV (% of VC)	Relative FEV[c]
26–33	Mean	72 2	165 0	5 57	105 6	85 1	106 9
	S D	9 0	22 9	0 64	8 9	5 4	
43–50	Mean	54 3	140 2	5 15	105 3	83 3	111 5
	S D	8 6	22 3	0 61	10 2	5 1	
58–64	Mean	44 2	137 2	4 39	97 9	81 9	118 9
	S D	6 3	20 2	0 48	8 9	4 3	

[a] Indirect measurement (3)
[b] Per cent of normal mean V_{O_2} max according to Åstrand and Ryhming (3)
[c] Per cent of normal mean VC and FEV (corrected for age and body dimensions) according to Berglund et al (6)

man hours of cross country skiing observed in Finland is still 4 times that expected in a comparable population not undertaking strenuous exercise (27) In addition several cases of sudden death have been observed in recent years in presumably healthy middle aged cross country skiers during ski contests in Norway Thus strenuous exercise may have deleterious effects even in extremely well trained middle aged men

The age specific prevalence of significant ischaemic ECG changes during exercise was similar to that found in presumably healthy sedentary middle aged males in our department showing an increased prevalence with increasing age (11) The present findings also compare favourably with the findings of Grimby et al in Swedish orienteering competitors aged 52–56 years (15) Thus hard physical training possibly may not protect against coronary atheromatosis provided the same parallelism between the ECG changes and coronary angiographic findings is found in these men as in apparently healthy sedentary men (11 12) In these asymptomatic sedentary men a positive exercise ECG was strongly and positively correlated to significantly positive coronary angiographic findings (12) In a recent study on 29 middle aged well ined asymptomatic cross country skiers as , , as 5 were not allowed to participate in strenuous cross country competitions due o serious arrhythmias and/or ischaemic ECG changes observed during a similar exercise test All these and the present men were thought to be in excellent health and had an authorized medical certificate for participating in such ski races (13)

The mandatory medical examination before being allowed to participate in such contests—which does not include exercise ECG testing—is obviously insufficient for detecting individuals prone to develop serious complications during strenuous exercise

As might be expected our selection procedures ruled out individuals with significant angina pectoris Whether skiers with angina are participating with less success in such skiing contests is unknown It is however noteworthy that some 3 5% of 2000 40–59-year-old presumably CHD-free subjects in a recent study in Oslo (11) had typical angina pectoris

The observed high prevalence of sinus bradycardia and various blocks of the conducting system is probably due to the increased vagal tone found in athletes (4 6 15, 25)—related to long periods of intense training This training also probably is responsible for the high prevalence of incomplete RBBB and voltage signs of left ventricular hypertrophy As expected these signs were far more prevalent in young than in elderly individuals and should not be considered signs of heart disease

The present observation of very high oxygen uptake in all three age groups compares favourably with previous direct measurements in well trained cross country skiers (4) A direct comparison among the various age groups should however be made with caution due to the differences in participation rate—even though this difference does not reach conventional statistical levels of significance If the observed heart rate particularly in the youngest age group represents their true (or very near true) maximal heart rate the indirect way of estimating the oxygen consumption should overestimate the true Vo_2 max (3) Still the maximal load on which the participants were able to perform exercise for 3 full minutes (data not presented) indicates that the presented estimates of Vo_2 max were fairly correct The figures for group III for instance correspond to the aerobic capacity of 20–30-years-old healthy male Norwegian students (4)

The lipid lowering effect of training is well in line with previous experience (19 20 21 23)

Disregarding the exercise ECG findings a typical cross country skier may be described as a lean extremely well trained non smoker with normal BP low resting heart rate and low lipid levels According to the general concept of CHD risk factors one should expect a low CHD morbidity and mortality in such men The evidence for such a view is conjectural although recent figures from one Norwegian study (26) indicate that CHD mortality in men participating in regular training is far less than in the normal population However several biasing factors may operate in the selection of individuals for regular training and therefore one ought to be cautious in extrapolating from such figures

In particular one should be cautious according to the discussion mentioned above on resting and exercise ECG findings This of course does not influence other positive effects of training such as an increased feeling of well being and an increased level of physical fitness which may be favourable during stress of various kinds

This study has made a case for further investigations of middle aged men wanting to participate in heavy training and competitive sports

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Smoking, Lung Function, Physical Performance and Latent Coronary Heart Disease in Presumably Healthy Middle-Aged Men

Jan Eriksen and Sven Chr Enger

From Medical Department B Rikshospitalet Oslo and the Department of Clinical Chemistry Drammen Hospital Drammen Norway

ABSTRACT During a cardiovascular survey comprising 2014 presumably healthy men aged 40–59 years latent (previously undetected) coronary heart disease (CHD), lung function and physical performance were related to differences in smoking habits The survey examination classified 1 832 individuals as "normals" (without clinical signs/symptoms of CHD) Among the others a strong suspicion of CHD was found in 115, of whom 105 had diagnostic coronary angiography No angiography was performed in another 35 individuals with slight albeit typical angina pectoris The remaining 42 men were excluded from this presentation for various reasons The following findings were obtained 1) In the 69 men with positive coronary angiography, the extent of coronary atheromatosis was positively related to the number of cigarettes smoked 2) The smoking habits of the 35 individuals with slight angina pectoris but no angiography did not differ from those of the "normals" 3) Physical performance during a near maximal bicycle exercise test and lung function according to spirometry were strongly and negatively related to smoking (in "normals") 4) Previous smokers and never smokers among "normals" had almost identical lung function and physical performance

Smoking is strongly associated with respiratory disease (34 37) However more needs to be known about the quantitative aspects of smoking different amounts of tobacco and about the possible beneficial effects of giving up smoking

Myocardial infarction (MI) and sudden unexpected death are strongly associated with smoking in most studies (34 37) but not in all (22 23 32) whereas little is published on the crucial issue Does smoking increase atherogenesis or does it only play an important part in the evolution of severe complications once severe coronary artery disease is established? Animal studies (2 3 5) can not be extrapolated to man and autopsy data conflict (4 8 35)

The aims of the present study were to relate differences in smoking habits in presumably healthy middle aged men to (*a*) Respiratory function and physical fitness as assessed by spirometry and a near maximal bicycle exercise test with particular emphasis on the effects of quitting smoking (*b*) Latent coronary heart disease (CHD) (the distinction between true and false positive suspect cases of CHD being made by means of coronary angiography)

STUDY POPULATION AND METHODS

Eighty six percent ($n=2014$) of all invited presumably healthy males aged 40–59 years from five major companies/governmental institutions in Oslo Norway participated in a cardiovascular survey with the primary intent of disclosing the presence/absence of latent CHD (12 13) All were accepted provided none of the following diseases/disorders were present Known CHD (MI diagnosed in hospital or angina pectoris diagnosed prior to the study by any physician) other known heart disease hypertension under treatment with drugs diabetes mellitus malignancy disorders of the locomotor system preventing a near maximal bicycle exercise test and miscellaneous disorders (12 14) The examination started at 7 30 a m after at least 12 hours fasting and abstention from smoking

The case history included the World Health Organization and the Greater New York Health Insurance Plan Survey questionnaires on angina pectoris (WHO Q and NY Q) (16 31) and a detailed history of smoking habits The 62 pipe or cigar smokers were included as cigarette

Table VI *Distribution of non smokers and current smokers in the clinical subgroups of presumably healthy middle aged men with and without latent coronary heart disease*

χ^2 for the distribution of smokers vs non-smokers among the 4 angiographied subgroups (normal angiography one vessel disease etc) is 16 51 (d f =3) i e $p<0\ 001$

Subgroup	No of individuals	Current non smokers		Current smokers	
		n	%	*n*	%
Normals	1 832	1 023	55 8	809	44 2
Non-angiographied individuals with angina pectoris	35	25	71 4	10	28 6
Individuals with suspect latent CHD but with normal coronary angiograms	36	24	66 7	12	33 3
Individuals with suspect latent CHD and positive coronary angiograms	*69*	*34*	*49 3*	*35*	*50 7*
Positive angiography and one vessel disease	18	14	77 8	4	22 2
Positive angiography and two-vessel disease	25	14	56 0	11	44 0
Positive angiography and three vessel disease	26	6	23 1	20	76 9

* 13 (50 %) smoked 10 cig /day in contrast to 500 (27 3 %) of 1 832 in the total group of "normals

eases Such knowledge may invalidate data from for instance clinical CHD materials

Concerning the validity of our data in latent CHD the findings should be representative for this particular group since 95% of those eligible for coronary angiography underwent this procedure By applying this procedure the diagnoses of CHD were made as early as feasible in apparently healthy subjects In individuals labelled normals a com sive non invasive technique including case ory clinical examination and exercise ECG test was used for excluding CHD (12 14) Still a udy like this inevitably misclassifies several individuals with advanced coronary artery disease as normals Thus autopsy studies in young men dying violently have shown advanced coronary atheromatosis in a considerable number of cases (11 25) Such misclassifications may obscure true associations However objections of this kind should be less valid in the present study than in studies applying less objective diagnostic measures than coronary angiography

a) *Pulmonary function in normals in relation to smoking habits*

The tests applied in the present study confirm the deleterious effects of smoking on lung function Whereas FVC FVC 1 0 sec and PEF differ in significantly in some studies in young smokers these smokers still have reduced lung function when N_2 wash-out curves closing volume and diffusing capacity are studied (7 17) In our middle aged subjects even the crude tests used showed a highly significant decrease in lung function in smokers and this decrease was directly related to the amount of cigarettes smoked Indeed the heaviest smokers had a lung function equivalent to non smokers 10 years older

b) *Physical performance in normals in relation to smoking habits*

The ability to perform work on the bicycle as expressed by the cumulative work and maximal work load tolerated also indicated a highly significant reduction of physical performance in smokers which was also directly related to the number of cigarettes smoked Since at least 12 hours had lapsed since the smokers had had their last cigarette the difference in physical performance between smokers and non smokers should not be due to the well known carbon monoxide effects on haemoglobin/myoglobin dissociation curves However the observed lower performance may not necessarily be due to the smoking as such Thus it has been shown that smokers in general have a more negative attitude towards physical activity than non smokers (6 10 19 24)

c) *Effects on a) and b) of quitting smoking*

All data from the present material indicate a negligible difference in lung function/physical performance in quitters and never smokers *Since* we have no longitudinal studies in individuals during and after cessation of smoking these data are only suggestive However recent longitudinal studies have convincingly shown that smokers who

qu t or reduce the amount of c garettes smoked mostly have a rap d and considerable improvement of several lung parameters (1 7 17 26) Thus at least a cons derable proport on of the impa rment of lung funct on n smokers seems to be due to re vers ble pathoanatom c changes in the lungs even in subjects who have been smok ng for years (7 26) Our results are in concert with these f nd ngs and should be encouraging for subjects who th nk of qu tt ng smok ng

d) Smok ng and latent coronary l eart d sease

It s only in the angiographied group that we know the extent of coronary atheromatosis and thus only th group should be used for assessing the relat on between smoking and atheromatos s W th n the angiograph ed group there was a highly s gn f cant pos t ve associat on between present smoking hab ts and the extent of coronary atheromatos s Th s m ght seem to corroborate the theory of a CHD-promot ng effect of smok ng However only nd v duals with three vessel d sease smoked s gn f cantly more than the average among ind v duals labelled normals

Prev ously we have shown that the ang ograph c f nd ngs were pos t vely associated w th the l p d levels (15) Thus in the present study ind v duals w th normal coronary ang ograms and one vessel d sease smoked the least and had the lowest l p d levels and v ce versa S nce we have also shown that smok ng increases l p d levels (13 15) one m ght argue that th s h gher level of cholesterol in th ee vessel d sease subjects was caused by smok ng However the l pid increasing effect of smoking s small (13 15) and therefore d fferences in smok ng hab ts *cannot expla n the d fferences in l p d* levels n the angiograph c subgroups Thus it seems reasonable to assume that the comb nat on of high l p ds and smok ng in some way is part cularly malignant for the evolution of coronary ather omatosis as also suggested previously (33) Thus hypothes s m ght expla n some confl ct ng data on the relat on between CHD and smok ng The papers report ng no associat on between CHD and smok ng are in general derived from populations n wh ch the mean populat on level of cholesterol is low compared with West European and US standards (22 23 30 32 35) In order to obtain the best information on the importance of smoking in ath erogenes s the comb ned effect of smok ng and cholesterol should be cons dered

The general impress on from the pre ent data is that smok ng plays an important part bu hat other factors may be as important for prom n or pro tecting from CHD in the ind vidual ca e l s for instance seen that a number of smok nt nu cual with h gh l p ds accord ng to nternat onal tanda d (15) have completely normal coronary rte e e en though they have smoked for years

The lack of assoc at on between r n nd ang na pectoris in our non ang o raph ec du als with ang na pectoris is d ff ul o pla n although the lack of ang ograph c ev d e e en further speculat on However th s f n rg n ac cord with several previous reports and th m y be one clue to the op n on th t ang na pe ori nd myocard al nfarct on/sudden unexpected d a h are not necessarily random expre on of the re disease (9 18 21 27 28 29 32 36

In conclusion the present data nd cate tha r ing is positively associated with decreased lun tun t on and physical performance whereas nd dua who had qu t smoking had a lung funct on and phys ical performance s m lar to never smokers The data also ind cate that smoking to some extent con tributes to atheroma format on n the coronary arteries but 1) that the magn tude of th s assoc a tion is uncerta n owing to the lack of an est mate of the number of false negative normals and 2) that smoking/h gh lipid levels may be a more s gn f cant factor than smoking alone

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Supraventricular Tachyarrhythmias in Acute Myocardial Infarction

R Lofmark and E Orinius[1]

From the Department of Medicine Karolinska Institute at Huddinge Hospital Huddinge Sweden

ABSTRACT Onset of atrial tachycardia flutter or fibrillation occurred in 11% of 274 consecutive patients with acute myocardial infarction (AMI) Atrial tachycardia started about 24 hours and atrial flutter/fibrillation about 72 hours after onset of AMI symptoms Left heart failure, diagnosed as pulmonary rales or frank pulmonary edema was not more common in these patients *before* onset of tachyarrhythmias than among the rest of the patients On the other hand, a notching of the P wave in lead CR_7 was significantly more common in the patients with atrial fibrillation (67%) In most of these cases the terminal P force in lead CR_1 was not negative as in so-called left atrial enlargement These findings suggest that atrial conduction disturbances might be a basis of atrial fibrillation in AMI

Atrial fibrillation flutter and tachycardia are common complicating arrhythmias in acute myocardial infarction (AMI) and they are nearly always transient or episodic (10 11) It has been suggested that these atrial tachyarrhythmias are due to atrial infarction/ischemia vagal reflexes or increased sympathoadrenal activity (3) Pericarditis has also been claimed to be a causative factor (6 7 9) The most widely held opinion seems to be that atrial tachyarrhythmias are due to pump failure especially left ventricular failure (8) This hypothesis will be reevaluated here in a consecutive series of AMI patients

PATIENTS AND METHODS

AMI was diagnosed during a two-year period in 274 patients at the Coronary Care Unit (CCU) of this hospital The diagnosis was based on central chest pain frank pulmonary edema or syncope *and* the development of pathological Q waves R progression or localized ST elevations in serial 12 lead ECGs *or* two or more elevated S-GOT (ASAT) values with a maximum at about 24 hours after onset of symptoms and higher than the S-GPT (ALAT) maximum *or* a myocardial necrosis at autopsy corresponding in age to the onset of symptoms

The mean age of these AMI patients was 65 years 41% of them were admitted less than 3 hours after the onset of symptoms The CCU stay lasted 69 hours on average During this period the ECG was continuously observed by the nurses on a central memory oscilloscope and they documented their diagnoses by cutting out strips from the 24-hourly recording of the monitored ECG During the CCU stay the lung fields were auscultated for rales at least 4 times a day The first appearance of any complication was registered in a special record as was the time which had elapsed from the onset of AMI symptoms until the occurrence of this complication

A finding of more than a few scattered basal rales indicated treatment with diuretics but not with digitalis However about one third of the patients had been on digitalis until admission to the CCU

A 12 lead ECG was recorded at least once daily during the CCU stay The last ECG was re-examined for notching of the P wave in the 6 chest leads ($CR_{4R, 1, 2, 4, 5, 7}$) *The criteria for the notch were that it should occur in most* of the recorded P waves in the lead concerned and in the same part of them

RESULTS

During the CCU stay *onset* of atrial fibrillation flutter or tachycardia was observed in 28 patients Atrial fibrillation was the first arrhythmia in 14 patients (5%) atrial flutter in 8 (3%) and atrial tachycardia in 6 (2%) (Table I) For comparison onset of ventricular tachycardia (3 or more consecutive ventricular premature beats) was noticed in

[1] *Present address* Department of Medicine Karolinska sjukhuset Stockholm Sweden

Table I *Observations during the stay in CCU*

	n	% of all AMI pats	Age (y) (mean ±S D)	% of pats with previous AMI	% of pats with treated arterial hypertension
Atrial fibrillation	14	5	70± 9	29	0
Atrial flutter	8	3	67±11	0	13
Atrial tachycardia	6	2	63± 6 *	17	17
Ventricular tachycardia (in patients without atrial fibrillation/flutter/tachycardia)	95	34	63±13	35	19

* $p<0\ 05$

95 patients without supraventricular tachyarrhythmias (34%)

The patients with atrial fibrillation but not those with atrial flutter or tachycardia were older than the controls with ventricular tachycardia Neither an anterior nor a diaphragmatic infarction site was overrepresented among the patients with supraventricular tachyarrhythmias

The onset of atrial as well as ventricular tachycardia was on average observed at about 24 hours after the onset of the AMI symptoms The mean delay in atrial fibrillation and atrial flutter was roughly 72 hours The atrial fibrillation flutter or tachycardia was preceded by pulmonary rales or frank pulmonary edema in 33–63% (mean 55) of the which does not differ significantly from the in ventricular tachycardia (Table II) or the of all the 274 AMI patients during the whole U stay

The study of the P wave revealed notching significantly more often in lead CR_7 in the atrial fibrillation group (67%) than in the ventricular tachycardia group or among all AMI patients (28 and 23% respectively) (Figs 1 and 2) This was not the case for the patients with atrial tachycardia and flutter (17 and 33% respectively) (Table II) The notching gave the P wave more or less an M configuration but only in 2 of the 6 fibrillation cases did its positive terminal force correspond to a negative phase in lead CR_1 No P wave had a duration of more than 0 12 sec The infarct site was as often anterior as diaphragmatic

In all but one of the 6 cases the P wave in CR_7 was notched before onset of atrial fibrillation In no one was a pre AMI ECG available Three of the 6 patients had had rales before the onset of atrial fibrillation A pericardial rub was observed in only one of the 6 patients with a notched P wave

Atrial damage from previous myocardial infarcts or arterial hypertension could be considered a contributory factor but in this series there were no differences between the groups in their incidences (Table I)

DISCUSSION

The incidences of atrial fibrillation flutter and tachycardia in the present series may seem low The explanation is that only *onset* of the *first* of the 3 atrial tachyarrhythmias was recorded while arrhythmias which had started before the admission or before the AMI were excluded as were

Table II *Observations during the stay in CCU*

	n	Site of AMI		S GOT maximum (U) (mean ± S D)	Hours between onset of AMI symptoms and arrhythmia (mean and range)	% of pats with rales or frank pulmonary edema before onset of arrhythmia	No of pats with notched P wave in CR_7
		Anterior	Diaphragmatic				
Atrial fibrillation	14	6	4	239±149	65 (4–206)	57	6/9
Atrial flutter	8	4	1	221±137	87 (9–192)	63	2/6
Atrial tachycardia	6	3	0	150± 73	23 (10–37) * *	33	1/6 *
Ventricular tachycardia	95	33	30	224±155	25 (1–301)	46	21/76

* $p<0\ 05$

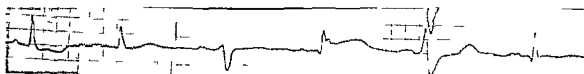


Fig 1 Lead CR_7 in 6 patients with atrial fibrillation

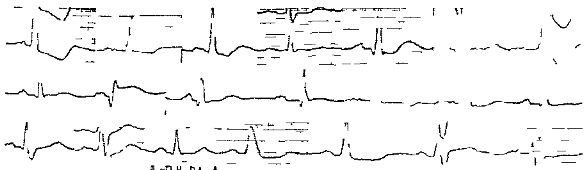


Fig 2 Lead CR_7 in 21 patients with ventricular tachy cardia

changes between fibrillation flutter and tachy cardia Even so 11% of the AMI patients were involved

The atrial tachyarrhythmias have been called the arrhythmias of pump failure in contrast to electrical instability arrhythmias as a label for ventricular tachyarrhythmias (8) Whereas left heart failure is certainly overrepresented in AMI patients with atrial fibrillation (2) left heart failure *before onset* of atrial fibrillation/flutter/tachycardia was not more common in the present series than among patients with ventricular tachycardia or all AMI patients Thus the hypothesis of left heart failure as the main cause of atrial tachyarrhythmias is not supported by this study

If one interprets the frequent P notching in CR_7 in this series as a manifestation of atrial conduction disturbances and regards conduction disturbances as a prerequisite for the re entry genesis of tachy arrhythmias this constitutes an hypothetical basis for atrial fibrillation in AMI Alternatively the P notching in CR_7 could be interpreted as a sign of left atrial enlargement but the lack of negative terminal force in CR_1 in most of the cases makes this interpretation less probable Moreover no cor relation has been found in AMI between the P wave terminal force in V_1 and left atrial dimension measured by echocardiography (12) Recently the ECG pattern termed left atr... shown to be unrelated to both left atrial size and pulmonary capillary wedge pressure but well cor related to a delay in atrial activation measured by endocardial mapping (4 5)

The P notching may be caused by atrial infarc tion although no significant relationship has been found in autopsy studies (1 13) between atrial in farct and atrial fibrillation in AMI Alternatively the P notching may have been present before the AMI and especially then some triggering mecha nism must be postulated e g increased vagal ac tivity

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Patients with High-Grade Atrioventricular Block Treated and not Treated with a Pacemaker

Ole Jorgen Ohm and Kjell Breivik

From Medical Department A University of Bergen School of Medicine and The Chr Michelsen Institute Department of Science and Technology Bergen Norway

ABSTRACT One hundred and eighty-one patients with chronic and with intermittent high grade atrioventricular block (AVB) were studied retrospectively Seventy-one were treated with fixed rate, 51 with demand type pacemaker, 59 were treated conservatively Observation time was three years minimum and 14 5 years maximum The mean age was about seven years higher, and 8 7% more patients had chronic AVB in the conservatively treated than in the pacemaker treated group About 12% more patients had Adams Stokes attacks in the pacemaker group than in the conservatively treated group There were 7% more patients with coronary heart disease (CHD) in the pacemaker group, and 10% more with aortic valve disease among the conservatively treated Totally 4 4% of the patients had a calcification of the mitral annulus fibrosus The two groups were comparable as regards functional class (NYHA) and heart size Survival showed 31% more sudden deaths in the conservatively treated than in the pacemaker group Sudden deaths were not more frequent among the patients with CHD than in those without Long term survival showed the more favourable result for the patients with than without pacemaker treatment There were eight (11 3%) unexplained sudden deaths among the patients treated with fixed rate pacemaker, only three (5 9%) among those treated with a demand unit The fixed rate and the demand units showed a mean longevity of 37 2 and 34 6 months respectively

The present paper is a retrospective study comparing conservatively treated and pacemaker treated patients with high grade atrioventricular block (AVB) admitted to Medical Department A Haukeland Sykehus in the 12 year period 1962–73

The intention of the study was to examine the etiology of heart block and the causes of sudden deaths related to heart block and type of pacemaker used Furthermore the choice of pacemaker for patients with high grade AVB is discussed Finally a comparison is made of the long term survival in the respective patient groups admitted during the years 1962–70

PATIENTS AND METHODS

Patients with congenital AVB (5) a single transient episode of AVB (5) leucemia (1) acute myocarditis (1) transient AVB due to acute myocardial infarction digitalis intoxication and electrolyte disturbances were regarded as irrelevant for this study

The study initially covered 183 patients (59 conservatively treated 124 pacemaker treated) Included were all consecutive patients with high grade AVB (second degree AVB with constant block usually leading to 2 1 3 1 AVB third degree AVB and atrial fibrillation with marked AVB resulting in a slow regular ventricular rhythm) of more than 24 hours duration The rhythm disturbance was documented by series of ECGs in all patients

Two patients in the pacemaker group were excluded from the study one because of pulse generator defect (fixed rate pacemaker) and one because of infection in the pacemaker pocket (QRS inhibited pacemaker) For the former patient it was decided not to replace the pulse generator because of mental reduction the latter seemed not to be pacemaker-dependent at the time of his infection and the pacemaker was removed

The study population was divided into two patient groups

Group 1 consisted of patients admitted to hospital during the period 1962–70 52 of whom were conservatively treated 56 pacemaker treated These 108 patients were analysed for long term survival A further eight patients who originally belonged to the group of conservatively treated patients later received a permanent pacemaker because of life threatening Adams Stokes attacks or cardiac failure In two of them the decision to implant a pacemaker and the later follow up took place in another hospital and they were therefore excluded from the study The remaining six were later added to the pacemaker treated patients in group 2 Four of them are still alive two have died (one of cerebral stroke one suddenly of unexplained cause)

Group 2 referring to the period 1971–73 consisted of the 52 conservatively treated patients from group 1 and

Table I *Details of the 181 patients included in the study*

Figures in parentheses indicate percentage

	Conservative treatment			Pacemaker treatment		
	Male	Female	Total	Male	Female	Total
No of pats	31	28	59	76	46	122
Age (y)						
Range	53–90	62–89	53–90	46–89	52–87	46–89
Mean	77 6	76.5	77 1	69 0	71 2	69.8
AVB-I	11	9	20 (33 8)	39	13	52 (42 6)
AVB-C	20	19	39 (66 1)	39	31	70 (57 4)
A S	23	17	40 (67 8)	58	39	97 (79.5)

[a] Intermittent [b] chronic AV block [c] Adams-Stokes attacks

seven new patients totalling 59 conservatively treated patients The reasons for not offering the seven new patients pacemaker treatment were advanced age and mental reduction in six patients all of whom died within a year (two unexplained sudden deaths one of cardiac insufficiency two of pneumonia one of pulmonary embolism) one patient who is still alive refused pacemaker treatment

Further group 2 comprised the 56 pacemaker treated patients from group 1 with 66 additional ones totalling 122 pacemaker treated patients The total patient material covering 181 patients was used for etiologic classification of heart block and causes of sudden death A certain number of the pacemaker-treated patients in group 2 was used for analysis of pulse generator longevity

of treatment

ı years of pacemaker implantation there were no criteria for the treatment but recurrent Adams-attacks and severe heart failure in spite of treatment with sympathomimetic drugs were regarded as an obvious indication for cardiac pacing

Fifty-nine patients were treated conservatively 47 with and 12 without sympathomimetic drugs One-hundred and twenty-two patients were treated with pacemakers (71 fixed-rate 46 QRS-inhibited 5 QRS-triggered) 82 of whom received sympathomimetic drugs before it was decided to treat them with a pacemaker

Details of the total study population of 181 patients are given in Table I The sex distribution shows a dominance of men in the pacemaker treated group whereas the number of men and women is almost the same in the conservatively treated group The mean age is more than seven years higher in the conservatively treated group indicating that there has been a selection by age The numbers of patients with constant and with intermittent high-grade AVB give approximately 9% more cases of intermittent heart block in the pacemaker treated group Adams-Stokes attacks are more frequent in the pacemaker-treated group 97 (79.5%) of 122 patients versus 40 (67 8%) of 59 patients in the conservatively treated

Table II *Etiology of heart block*

	Conservative treatment (59 pats)		Pacemaker treatment (122 pats)		Total (181 pats)	
	n	%	*n*	%	*n*	%
Primary	34	57 6	64	52.5	98	54 1
Coronary heart disease	9	15 3	27	22 1	36	19 9
Aortic valve disease	12	20 3	12	9.8	24	13.3
Calcification of mitral annulus fibrosus	3	5 1	5	4 1	8	4 4
Rheumatic heart disease (without valve disease)			3	2 5	3	1 7
Ankylosing spondylitis			4	3.3	4	2.2
Collagen disease			4	3 3	4	2 2
Myasthenia gravis			1	0 8	1	0 6
Gonorrheal myocarditis			1	0 8	1	0 6
Sarcoidosis	1	1 7			1	0 6
Hypertension			1	0.8	1	0 6



Fg 1 Chest X ray n the lateral v ew from a 74 year old woman *A heavy calc f* ation correspond ng to the m tral annulus fib *osus s clearly* ev dent There were no cl n cal man festa ons of m tral valve d sease ECG demonstrated trifasc ular *d sease* and *H s* bundle record ngs that the si e of block *was local zed d stal* to the bundle of H s The pa ent was treated *w th a permanent f xed* rate *pulse* genera or and a b polar endocard al electrode

group The number of pat ents w th interm ttent and w th ch on c AVB who experienced Adams Stokes attacks was 15 (25 4%) versus 38 (31 1%) and 25 (42 4%) versus 59 (48 4%) in the conservat vely and pacemaker treated groups respect vely

Fo low up nformat on was gathered through regular ontrols in the Outpat ent Cl n c or from other doctors who saw the pat ents regularly *All pat ents were followed* un 1 Dec 1976 or unt l death Informat on was collected o all the pat ents included n the study

Mo al ty

The analys s of death is based mostly on a descr pt on of the terminal cl n cal p cture but also on necrops es De tailed *stud es of the bundle branch system* vere not car ried out

In the conservat vely t eated group 41 (74 5%) of the 55 dea hs occurred in a health nst tut on and autopsy was performed n 20 (41 8%) of these pat ents In the pacemaker treated group 49 (81 6%) of 60 pat ents d ed n a health nst tut on autops es be ng performed n 27 (55%) of them These h gher f gures for the pacemaker t eated group nd cate that th s group rece ved better med cal care during the term nal part of l fe The h gher frequency of postmortem exam nat ons for th s group al o ind cates that the diagnoses were more sat sfactory

For the pat ents who d ed outs de ho p tal the d agnos s is based on the nformat on f om rela ves doctors who saw the pat ents and off a tat st cs o he c all d aths are class f ed acc rd ng to et

Et ology of heart block

A compa son of the causes of the AVB tween the two pat ent groups (Tab e II accord ng to et ology in l fe d ff c t e pr the primary AVB and AVB cau d by sclerosis of the coronary arteries no g n a unt l a term nal myocard l nfarc In group of coronary heart d ease (CHD w th ang na pectoris proven my c ECG changes nd cat ve of CHD

The dom nant et olog es are he 57 6% of the pat ents in the conse t e the pacemaker treated groups The e CHD of 22 1% among the pacemake n opposed to 15 3% among the con a whereas there is a dom nance of aort d 20 3% among the conservat vely trea ed g p 9 8% among the pacemaker treated

Of 23 pat ents w th aort c valve d sease l had pa 75 years of age and t s reasonable to attribute the va changes to degenerat ve calc f cat on (13) Pa en s v h calcif cat on of the m tral annulus f brosus w thou any valve affect on numbered e ght of those included n e study (4 4%) They were pat ents w th an age range of 71 85 years (mean 77 4) and showed a dom nance of wom n ($n=6$) over men ($n=2$) which has also been reported by Pomerance (12) S nce th s was a casual f nd ng at X ray exam nat on t is poss ble that th s group of pat ents was larger because calc f cat on nvolv ng small areas can be d ff cult to recogn ze rad ograph cally The close relat on between the annulus f brosus and the bundle branch system makes th s the most probable et olog c cause of the AVB (F g 1)

Three pat ents had a h story of prev ous rheumat c fever but w thout any subsequent valve d sease There s also a small percentage (3 3%) of pat ents w th ankylos ng spondyl t s Three of these four pat ents also had aort c valve d sease two of them w th serious hemodynam c consequences It should therefo e be quest oned whether the valve d sease was the cause of the r heart block

W th regard to hypertens on as an et olog c factor only pat ents w th a d astol c BP of >100 mmHg were class f ed as hav ng hypertens ve heart d sease Isolated elevated systol c BP caused by h gh stroke volumes n pat ents with bradycard as was not class f ed as the cause of heart block

Other m scellaneous causes of heart block we e col lagen d ease n four pat ents myasthen a grav s gon orrheal myocard t s and sarco dos s n one pat ent each

Funct onal class

Funct onal class accord ng to the New York Heart As soc at on (NYHA) and heart s ze were compared n the two groups mmed ately prior to treatment (Table III) There were a few more pat ents in funct onal class I among the pacemaker treated than among the conserva

Table III *Functional class and heart size in the two patient groups prior to treatment*

	Conservative treatment (59 pats)				Pacemaker treatment (122 pats)			
NYHA class	I	II	III	IV	I	II	III	IV
No of pats	7 (12%)	30 (50 8%)	19 (32 2%)	3 (5%)	25 (20 5%)	58 (47 6%)	35 (28 7%)	4 (3 2%)
Cardiac/thoracic ratio								
Range	0 44–0 68				0 41–0 76			
Mean	0 54 (n=46)				0 53 (n=119)			

tively treated patients Heart size expressed as the cardiac/thoracic ratio was nearly the same in the two groups

RESULTS

The mortality figures (Table IV) show that 55 patients (93 2%) have died and four (6 8%) are alive in the conservatively treated group versus 60 (49 2%) and 62 patients (50 8%) in the pacemaker group The majority of deaths are unexplained sudden deaths and cardiovascular deaths

The frequency of unexplained sudden deaths is far higher in the conservatively treated group amounting to 35% of the deaths If deaths from ventricular fibrillation and asystole are considered sudden the percentage rises to 52 5 (29/55) Together with the three deaths classified as unspecified cardiac the percentage is 58 2 (32/55)

On the other hand the number of unexplained sudden deaths is also high in the pacemaker treated group comprising 11 patients (18 3%) Including the cases who died from documented ventricular fibrillation and asystole the percentage of deaths is 26 7 (16/60) which is less than half the sudden deaths in the conservatively treated group

Three patients (2 5%) died in the postoperative period Two of them were among the first to be equipped with a pacemaker one of them having a serious myasthenia gravis In the second a temporary electrode was displaced during the permanent pacemaker implantation resulting in asystole and cerebral damage The third patient died in cardiac tamponade caused by perforation of an endocardial electrode

Deaths of non cardiac causes included pneumonia (8/8) carcinoma (3/7) advanced age (1/1) in the conservative and pacemaker group respectively In addition one patient died of pulmonary insufficiency one committed suicide one died of ileus one of peritonitis and one of septicemia unrelated to pacemaker therapy

Table IV *Causes of death*

	Conservative treatment	Pacemaker treatment	Total
Unexplained sudden	19	11	30
Cardiovascular	24	25	49
Myocardial infarction	3	6	9
Heart failure			
Acute	2	0	2
Chronic	2	5	7
Ventricular fibrillation	3	3	6
Asystole	7	2	9
Unspecified cardiac	3	0	3
Pulmonary embolism	1	3	4
Cerebral stroke	3	6	9
Postoperative		3	3
Non-cardiac	12	21	33
Total	55	60	115

Table V *Etiology of heart disease and type of heart block in relation to sudden death in the 181 patients studied*

AVB-C=chronic AVB I=intermittent AV block AVD=aortic valve disease CHD=coronary heart disease RHD=rheumatic heart disease CAF=calcified annulus fibrosus Primary=primary type of heart block

	Conservative treatment				Pacemaker treatment			
	AVB C		AVB I		AVB C		AVB I	
Unexplained sudden death	AVD Primary CHD Sarcoidosis	6 7 1 1	AVD Primary	1 3	AVD Primary CHD	1 1 1	AVD Primary CHD RHD	2 3 2 1
Asystole	Primary	6	Primary	1	Primary	1	CHD	1
Ventricular fibrillation	Primary	1	Primary	2	AVD	1	CHD	1
Unspecified cardiac	Primary CAF	1 1	Primary	1	Primary	1		
Total		24		8		6		10

DISCUSSION

The causes of heart block were similar to what has been described by others (4 6 15) Calcification of the annulus fibrosus mitralis as a cause of heart block has perhaps been underestimated and is more frequent than has been anticipated especially in the elderly (Table II Fig 1)

The dominance of CHD in the pacemaker treated group would be unfavourable for life expectancy Only five of these patients died suddenly (Table V) On the other hand preponderance of aortic valve disease in the conservatively treated group should be disadvantageous for that patient group Seven of these patients expired suddenly (Table V)

Among the unexplained sudden deaths in the pacemaker group we found eight patients treated with fixed rate and three with demand pacemaker Of the former five had *intermittent AVB* and a considerable degree of parasystole was recorded in six The subject of interference with spontaneous cardiac activity as a cause of sudden death is however a matter of great controversy Zoll and Weintraub (16) found in a series of 241 patients treated with fixed rate pacemakers that sudden death was less frequent among those with than without a competitive rhythm (24% versus 33%) Among our patients with fixed rate pacemaker 11 (15 5%) died suddenly However if two cases of sudden death obviously due to battery depletion are excluded the percentage is reduced to 12 7% Among our patients with demand pacemaker five (9 8%) died suddenly Although the number of patients is small our results cannot confirm those of Mascarenhas and Center (8) who noted no reduction in the incidence of sudden death among their patients treated with a demand pacemaker The discrepancy between these findings and ours may be due to the fact that there was a higher number of demand treated ($n=399$) compared with fixed rate treated ($n=32$) patients and that other rhythm disturbances than AVB were included in their study

Considering the patients who died suddenly it is interesting to note that among the pacemaker treated six had chronic and ten intermittent AVB whereas in the conservative group 24 out of 32 patients had chronic and only six intermittent AVB (Table V)

It seems impossible to predict which patients can live with AVB without pacemaker treatment Siddons (14) in an analysis of his material could not find any correlation between bundle branch block and sudden death The two youngest patients in the conservatively treated group (53 and 62 years old) died suddenly seven months and a few days after leaving hospital They both had chronic high grade AVB and one of them in addition a right bundle branch block whereas the other had QRS complex of normal duration The latter patient had however long standing sarcoidosis this being the most possible cause of the heart rhythm disturbance Four of six patients with probable sarcoidosis reported by Ghosh et al (5) died suddenly Although sarcoidosis is not a very common cause o[f he]art block such patients with a documen[ted]

rhythm disturbance or unexplained syncope should always be treated with permanent pacemaker On the other hand three of the survivors in the same group (86 86 and 94 years old) one of whom had a trifascicular disease have all had frequent Adams Stokes attacks

Of our total patient series 33 (47 1%) of 70 with chronic and 29 (55 8%) of 52 with intermittent AVB are still alive in the pacemaker treated group The corresponding figures in the conservatively treated group are two patients each (5 1% and 10%) This seems to support the statement of Johansson (6) that patients with intermittent block have a better prognosis than patients with chronic AVB

On the other hand Lichstein et al (7) concluded that decreasing block in pacemaker treated patients with trifascicular disease resulted in poorer survival They ascribed this to CHD Of the 36 patients with CHD in our series 24 (66 7%) have died The mortality is thus only slightly higher than for the whole material 63 5% (115/181) According to the data for the pacemaker treated group 16 (59 3%) of 27 patients with CHD have died The mortality for this group is thus lower than the mean for the whole series Our data also seem to give some support to the results of Lichstein et al (7) since 10 of the 16 patients with CHD who have died had intermittent AVB

ing view on pacemaker treatment

demand pacemaker was introduced in our de ment in 1969 but was not widely used until 1971 Together with the general acceptance of pacemaker treatment this is the main reason why the number of pacemaker patients increased so rapidly from that year on The number of patients on a conservative regimen increased by only seven (from 52 to 59) in the second period whereas the number of pacemaker treated patients more than doubled (from 56 to 122) In the first period all pacemaker treated patients received sympathomimetic drugs In the second period 1971–73 only 26 of 66 new pacemaker patients were tried on drugs This definitely demonstrates the tendency to perform a pacemaker implantation even in patients with less serious symptoms such as dizziness and dyspnea during physical activity and in a few cases also prophylactically While up to 1971 the percentage of patients in functional class I had been almost equal in the two groups the number of patients in the pacemaker group increased up to 1973 (Table III) On the other hand due to the availability of better equipment there has been a tendency to implant permanent pacemakers in patients also otherwise seriously ill

While patients with Adams Stokes attacks numbered 36 (69 2%) out of 52 and 54 (96 4%) out of 56 in the conservative and pacemaker groups respectively these data were unchanged for the conservatively treated group (67 8%) and reduced for the pacemaker group (79 5%) indicating a more liberal use of pacemakers (Table I) This was also true with regard to constant versus intermittent AVB in the pacemaker group during the two periods the percentage with chronic AVB dropping from 69 6 to 52 4

Selection of pacemaker

We still recommend fixed rate pacemaker in patients with chronic high grade AVB In this series we had to change from fixed rate to demand pacemaker in only two patients due to interference with spontaneous heart rhythm In two patients we changed from demand to fixed rate pacemaker since the patients had no spontaneous rhythm during follow up

During 1969–73 36 patients were treated with fixed rate pacemaker Twenty six of them had no spontaneous heart activity during follow up four had significant and six insignificant competition between pacemaker and spontaneous activity Among 52 patients with demand pacemakers 29 had much spontaneous activity whereas 16 had none In seven of these patients observations were lacking It seems thus that a more liberal use of fixed rate pacemaker is to be recommended during pulse generator replacement

In the total series for the years 1970–73 comprising a total of 115 new implantations and 59 replacements the use of fixed rate pacemakers during new implantations dropped from 37 5 to 16 3% Including replacements the corresponding figures are 72 7 and 18 6% This mostly has to do with the fact that increasing numbers of patients with other arrhythmias than AVB are treated with pacemaker The sinus node rhythm disturbances leading to a permanent implantation have from 1973 on been almost equal in number to the AVB in our entire patient material (10) There is also a marked tendency to use demand pacemakers in patients with high grade AVB to avoid competitive rhythms which may occur even in such patients However

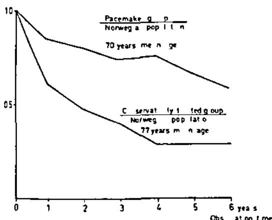


Fig 2 Sex adjusted survival in the period 1962–70 when the number of pacemaker treated and conservatively treated patients was almost equal 56 and 52 respectively The mean age was the same as for the total series

there would then be the risk that patients with a high grade AVB and with a slow or absent idioventricular rhythm might have new Adams Stokes attacks and prolonged asystole if the pacemaker was inhibited by external interference On the other hand the risk of this occurrence has diminished due to better shielding of the pulse generator and conversion to an interference rate

The major advantages of the fixed rate pacemakers over the demand pacemakers have been their lower cost less complexity greater reliability and longevity Since energy sources and electronic circuits have undergone continuous improvement there is little point in comparing pulse generator longevity from the earliest years with that of recent years Considering our patients and comparing the fixed rate and the demand pulse generators replaced in the 5 year period 1972–76 we found a mean longevity of 37 2 months (range 18–63) in 35 fixed rate pulse generators while in 34 demand units the mean longevity was 34 6 months (range 9–57) All pulse generators were powered with mercury-zinc cells However factors such as different pulse widths pulse amplitudes and electrode areas which are of major importance for current drain were not compared for the different units Excluded from these data were also some pulse generators belonging to certain series showing early pacemaker malfunctions due to electronic component defects and/or early battery failures which have occurred lately In our patient group almost 9% of new implantations and replacements in the 3 year period 1974–76 with a dominance for demand pacemakers belonged to these series Parsonnet (11) has found an even greater number of early malfunctions 6% per year

Long term survival

Several studies have been published on survival of patients with high grade AVB (1 4 6 14) Edhag (4) and Johansson (6) have clearly documented the better prognosis with a pacemaker as opposed to conservative treatment Siddons (14) found a survival of patients almost similar to that of the general population after the first year of observation Amikam et al (1) have shown that pacemaker treatment prolongs life even in the most advanced age groups

From 1962 to 1970 we had 52 patients treated conservatively and 56 with pacemaker (9) With the exception of age mental reduction Adams Stokes attacks and a few patients who refused pacemaker treatment there were no specific selection criteria Studying the expectation of life data for these two patient groups (3) now having been followed for a minimum period of six years (Dec 1976) it is clear that the survival shows the more favourable result in the pacemaker treated group (Fig 2) A more definite conclusion can be drawn when all these patients have been followed until death

CONCLUSION

Unfortunately no prospective study has been undertaken which could answer the question whether pacing prolongs life in patients with high grade AVB Based on this retrospective study it is reasonable to conclude that patients with high grade AVB should be treated with pacemaker Excluding patients with heart block caused by Chagas disease reported from certain Latin American countries (2) the etiologies of heart block are roughly the same in the industrialized parts of the world Better equipment and simpler operative technique have reduced the rate of complications and will improve the survival rate It would be difficult to undertake a prospective randomized study today in patients showing symptoms that might indicate AVB knowing the benefit and better quality of life in these patients if they are treated with pacemaker In patients with high grade AVB and lacking spontaneous cardiac activity fixed rate pacemakers shoul

still be used However in our material the mean longevity of the fixed rate units was only 2 6 months more than for the demand units The wider use of hermetically sealed lithium powered pace makers with an anticipated lifetime of perhaps more than ten years even for demand units will favour the use of these pacemakers

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Left Anterior Hemiblock in Acute Myocardial Infarction

Incidence and Clinical Significance in Relation to the Presence of Bundle Branch Block and to the Absence of Intraventricular Conduction Defects

Jan Erik Otterstad Stein Gundersen and Norman Anderssen

From the Medical Department Telemark Central Hospital Skien Norway

ABSTRACT The incidence of intraventricular conduction defects was examined retrospectively in 449 consecutive patients with acute myocardial infarction (AMI) The incidence of left anterior hemiblock (LAH), right bundle branch block (RBBB), left bundle branch block (LBBB) and RBBB+LAH was 12 2, 4 2 3 8 and 2 5%, respectively At least 24 patients (5 8%) developed LAH as a result of the AMI LAH was present in 20% (33/164) of patients with anterior infarction, in 14% (18/131) of those with infarction of undetermined localization and in 3% (4/143) of patients with diaphragmatic infarction The incidence of complete atrioventricular (AV) block in patients with LAH was 6% and in patients with no intraventricular conduction defects 7% In patients with RBBB RBBB+LAH and LBBB, the incidence of complete AV block was 37, 45 and 18% respectively Severe pump failure occurred with the same low incidence in patients with LAH as in patients without intraventricular conduction defects but was much more common in patients with complete bundle branch block (BBB) The mortality rate for patients with LAH was 22% and for patients with no intraventricular conduction defects 21% In patients with RBBB RBBB+LAH and LBBB, the mortality rates were 53 55 and 53%, respectively Patients with complete BBB had a higher age and a higher incidence of previous AMI than the others Compared to patients with no intraventricular conduction defects, the presence of LAH did not increase the mortality rate or the risk of developing severe heart failure or complete AV block in contrast to the serious prognosis in patients with complete BBB

The term left anterior hemiblock (LAH) was introduced by Rosenbaum et al (17) and is meant to imply left axis deviation due to block in the anterior division of the left bundle branch The presence of LAH in acute myocardial infarction (AMI) has been discussed in connection with the short term prognosis and development towards total AV block especially when LAH is combined with right bundle branch block (RBBB) Right or left bundle branch block (LBBB) in AMI is associated with an increased tendency towards development of complete AV block or pump failure and carries a poor short term prognosis (3 7)

The purpose of this study was to compare AMI patients with LAH to patients with bundle branch block (BBB) and patients with no intraventricular conduction defects and thus establish the incidence and clinical significance of LAH in AMI

PATIENTS AND METHODS

The study comprises 449 consecutive patients treated in the Coronary Care Unit (CCU) Patients with acute onset of chest pain left ventricular failure arrhythmia with or without concomitant chest pain and in some cases unexplained syncope or circulatory arrest were admitted to this unit A diagnosis of AMI was made in accordance with WHO standards when two of the following criteria were fulfilled 1) Typical history of chest pain or sudden development of left ventricular failure 2) Typical ECG changes with development of diagnostic Q waves 3) Characteristic elevation of s ASAT Patients who did not fulfill two of these criteria and patients with a delay of more than 48 hours between onset of symptoms and hospitalization were excluded All patients are represented once only i e readmission in the period of investigation is not included Age range mean age and sex distribution are listed in Table I

Our diagnostic criteria for LAH were according to Rosenbaum et al (17) Left axis deviation to the left of −45 with a QRS duration of less than 0 11 sec q in I and aVL rS in II and III and R in I Fifty five patients fulfilling these criteria form group A In no case could a certain diagnosis of left posterior hemiblock be made according to Rosenbaum s criteria

Our criteria for BBB were according to Godman (6)

Table IV *Localization of infarctions in patients with LAH RBBB RBBB+LAH LBBB and with no intraventricular conduction defects*

LAH=left anterior hemiblock RBBB=right bundle branch block LBBB=left bundle branch block

	Anterior		Diaphragmatic		Indefinite		Anterior + diaphragmatic		Total	
	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)
LAH	33	60	4	7	18	33			55	100
RBBB	10	53	5	26	3	16	1	5	19	100
RBBB + LAH	10	91	1	9	0				11	100
LBBB	*3*	*18*	*2*	*12*	*12*	70			*17*	100
No intraventricular conduction defects	108	31	131	38	98	28	10	3	347	100
Total	164	37	143	32	131	29	11	2	449	100

day and one survived without serious complications Neither developed total AV block In no case did development of LAH after admission proceed *to pure LBBB*

Table IV shows that 60% (33/55) of patients with LAH had anterior and 7% (4/55) diaphragmatic infarctions The corresponding figures for patients with no intraventricular conduction defect are 31% (108/347) and 38% (131/347) respectively The incidence of infarctions with indefinite localization is similar in the two groups 33% for patients with LAH and 28% for patients with no intraventricular conduction defect Ninety-one % (10/11) of patients with RBBB+LAH had anterior infarctions com to 53% (10/19) of patients with RBBB and axis Only 9% (1/11) of patients with +LAH had diaphragmatic infarction com d to 26% (5/19) of patients with RBBB and a axis

Totally an isolated finding of LAH was found in 20% (33/164) of patients with anterior infarction and in 3% (4/143) of patients with diaphragmatic infarction

Patients in group B had a somewhat higher incidence of supraventricular tachyarrhythmias ventricular tachycardia and ventricular fibrillation compared to groups A and C but the differences were not statistically significant

DISCUSSION

The incidence of 12 2% for LAH in this study among patients with AMI is comparable to that of some series (2 3 13 19) but higher than in others (10 18) Selection criteria and frequency of ECG registration during the acute course of AMI may explain these variations The minimum incidence of 5 8% for LAH caused by the acute infarction in our series is in accordance with that of other authors (3 16) *The patients with irreversible LAH on admission* can most probably be divided into two subgroups as proposed by Rizzon et al (16) a larger group with preexisting LAH in most cases associated with previous ischaemic heart disease (1 9) and a smaller number of patients who develop LAH after onset of AMI but before admission to hospital

The occurrence of complete AV block was the same in patients with LAH as in patients with no intraventricular conduction block a finding comparable to other series (2 3 8 10 13 19) This observation has been explained by referring to the widely accepted concept of a bidivisional left bundle branch asserting that the left anterior division is a thin delicate structure with blood supply from the left anterior descending artery (LAD) and the posterior division a more solid structure with blood supply from the right coronary artery (RCA) Interruption of conduction in the anterior division could therefore be caused by a small infarction leaving the posterior division as well as the right bundle unharmed Recent anatomical studies (4 14) do not verify the simple model of the left bundle branch with two hemidivisions Its structure is rather diffuse with several branches richly anastomosing (14) Accordingly small lesions in anterior superior parts of the septum should not cause a significant conduction delay owing to the presence of the anastomoses A shift of axis may take place however due to a relatively delayed activation of anterior superior parts of the left ventricular myocardium with respect to posterior and basal

parts Another mechanism for the LAH pattern in AMI would be peri infarction block (14) or a large anteroseptal infarction If the latter mechanisms were the main causes of LAH in AMI one would expect a poorer prognosis because relatively large infarctions would then be necessary to produce the shift of axis to the left The short term prognosis in patients with LAH in our series did not differ from that of patients with no intraventricular conduction defects a finding comparable to most other series (? 3 8 13 16 19) This suggests that a combination of LAH with mostly large infarctions is unlikely

The incidence of total AV block in patients with RBBB+LAH and patients with RBBB and a normal axis was nearly identical Scheinman and Brenman (18) reported similar results but their figures were only 13% and 17% respectively compared to 45 and 37% in our series Lie et al (11) however found progression to total AV block in 7 of 18 patients with RBBB+LAH complicating anteroseptal infarction compared to none of 18 patients with RBBB and a normal axis On the other hand Godman et al (7) found that 31% of patients with RBBB developed total AV block but they gave no information about the mean frontal axis In a survey of recent literature Mullins and Atkins (15) found a mean incidence of total AV block of 46% in patients with RBBB+LAH and of 43% in patients with RBBB all suffering AMI Thus the question whether LAH combined with RBBB in AMI increases the risk of developing complete AV block compared to RBBB and a normal axis seems to be unsettled

The relatively low incidence of total AV block of 18% in patients with LBBB is surprising though it agrees with the findings of others (15) and is difficult to explain

In only 2 patients in the present series was LAH followed by LBBB Both had LAH on admission and a history of symptoms of only 4–6 hours and the LAH may have been preexistent Progression of LAH to LBBB in AMI seems to be very unusual The reason for this is probably the dual blood supply to the left bundle branch In keeping with this patients with LBBB due to arteriosclerotic heart disease often have triple vessel disease (3) Occlusion of one artery may besides causing infarction in its own area of supply reduce collateral blood flow to other stenotic arteries For instance occlusion of the LAD may cause anterior

also reduce collateral blood flow to a stenotic RCA Such a mechanism could explain the sudden onset of LBBB in AMI or the development of LAH followed by LBBB The latter event could easily be missed due to the short interval involved and because of too infrequent ECG registrations LBBB could also be produced in AMI by more peripheral lesions of the left bundle by affecting the anterior and posterior divisions separately ECG evidence of this would be the intermittent appearance of left anterior hemiblock and left posterior hemiblock preceding complete LBBB According to Col and Weinberg (3) such a case has never been described

The most frequent localization of infarction in patients with LAH was anterior and consistent with the assumption that LAH is associated with disease of the penetrating branches of the LAD These also supply the right bundle with blood (5) and accordingly 10 of 11 patients with RBBB+LAH had anterior infarctions Nine of these developed RBBB during infarction a slightly higher figure than the 6 patients who developed LAH (Table III) If the LAH was mainly due to peri infarction block in anterolateral parts of the left ventricle an opposite tendency might have been expected but no conclusions can be drawn from these small figures

The sporadic presence of LAH either isolated or combined with RBBB in diaphragmatic infarctions can be explained by deprivation of collateral blood supply to a stenotic LAD due to occlusion of the RCA (16)

The incidence of severe heart failure in patients with BBB was 36% and approximately 4 times as high as in the rest of the patients a finding comparable with findings by others (18) The presence of LAH did not increase the tendency to severe heart failure compared to patients with no intraventricular conduction defects although the LAH group had a greater proportion of patients with enlarged hearts and of patients being treated with digitalis on admission

The patients with BBB had a higher mean age and a higher incidence of previous infarctions than the other patients and therefore probably ran a greater risk of developing complications In addition to the high incidence of complete AV block and of severe heart failure the patients with BBB also had by far the highest mortality rate The high mortality rate in patients with AMI and BBB has also been reported by others (15 18) but contradictory findings do

CONCLUSION

The presence of LAH in AMI was a relatively fre quent finding and was not a significant risk factor with regard to development of total AV block se vere pump failure or mortality compared to patients with no intraventricular conduction block In con trast the presence of complete BBB was combined with a higher mean age incidence of recurrent in farction as well as a significantly increased risk of developing total AV block and severe pump failure and carried a higher mortality compared to patients with LAH and no intraventricular conduction block

The combination of RBBB and LAH was not associated with a higher incidence of total AV block in relation to RBBB and a normal axis Temporary appearance of LAH preceding development of LBBB could not be registered for certain but 2 pa tients who later in the course of AMI developed LBBB had LAH on admission

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Thrombosis of the Inferior Vena Cava, Disseminated Intravascular Coagulation and Gangrene of the Penis

A Case Report

Gunnar Sødal Bernt Ly and Hans Henrik Chr Borchgrevink

From Surgical Department B Medical Department A Section of Haematology and Department of Plastic Surgery Rikshospitalet Oslo Norway

ABSTRACT Gangrene of the penis following thrombosis of the lower segment of vena cava inferior is reported in a patient who also exhibited clinical and laboratory signs of disseminated intravascular coagulation Development of the gangrene may have been related to thrombotic occlusion of the right spermatic vein

Although known for many years (10 11) thrombotic obstruction of the inferior vena cava remains an uncommon clinical syndrome Its clinical features depend on the level completeness and rapidity of the obstruction as well as on any underlying disease (1 6 12) Obstruction can occur in any portion of the vein but is usually caudal to the renal veins (11) In a recent survey (12) comprising 64 cases the most common underlying causes were carcinoma of the kidney postoperative deep vein thrombosis and carcinoma of the pancreas in a significant number of cases the underlying cause remained obscure (12)

The syndrome has also been associated with hypercoagulable states (6) but disseminated intravascular coagulation (DIC) has usually not been detected Gangrene of the legs may occur (3 8 11) and is probably related to widespread venous thrombosis of the lower extremities Because gangrene of the penis does not seem to have been reported in vena cava inferior thrombosis we wanted to present the following case

CASE REPORT

The patient a 35 year-old previously healthy tunnel worker was admitted to a local hospital with a fracture of the left lateral malleolus Osteosynthesis was performed 1 week later Fifteen days after the injury he complained of pain in his left thigh and during the subsequent days he developed gross *pitting edema of both legs* *Left* sided ascending phlebography five weeks later failed to demonstrate filling of the deep veins Treatment with warfarin was then started

Three days later however he developed massive edema of the penis and scrotum with impending gangrene of the penis and was transferred to Rikshospitalet Oslo On admission he was pale and exhausted and complained of abdominal pain There was massive pitting edema of both legs and the lower parts of abdomen In the lateral regions of the abdomen there were extensive collateral superficial veins with a cranial direction of blood flow There was massive edema of the penis and scrotum with impending ganrene of the penis (Fig 1*a*) Most of the superficial veins of both arms were thrombosed The respiration was normal and the BP 130/70 mmHg He was able to pass urine but in small portions only

Laboratory assays HB was 8 5 g/dl WBC 15 000/μl thrombocytes 208 000/μl and ESR 60 mm/h Serum iron was 2 5 and TIBC 34 μmol/l Serum sodium and chloride were markedly depressed (117 and 85 mmol/l respectively) and serum potassium moderately elevated (5 5 mmol/l) Serum glucose creatinine albumin bilirubin and calcium concentrations blood gases and acid–base values ALP ASAT ALAT LDH thoracic X ray and ECG were normal

Retrograde angiography of the inferior vena cava demonstrated total occlusion at the level of the renal veins Selective renal venography revealed two open renal veins on the right side whereas the left renal vein could not be visualized

Coagulation assays (Table I) demonstrated a positive ethanol gelation test and high values of fibrinogen/fibrin degradation products (FDP) interpreted to indicate DIC As shown large doses of heparin were needed to double the activated partial thromboplastin time (Cephotest) thus indicating increased heparin tolerance

Treatment The patient was heparinized and given vita

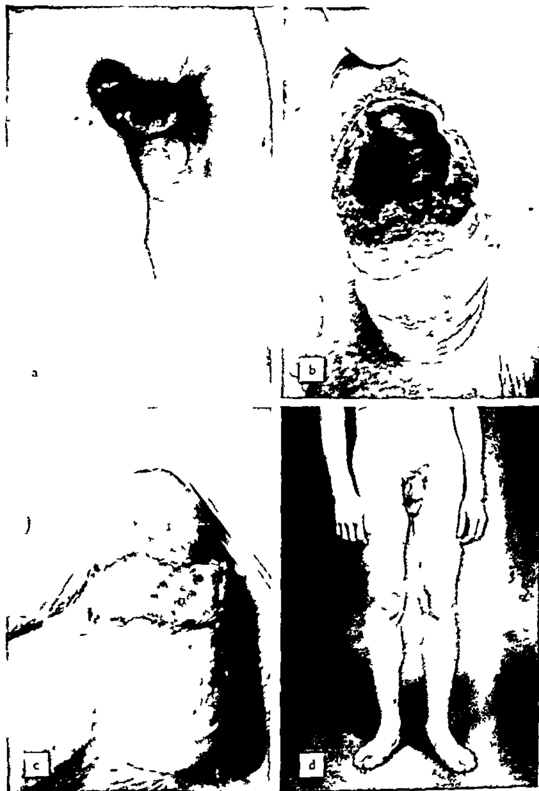


Fig 1 Pictures taken on admiss on (*a*) before and after skin grafting (*b* and *c*) and three months later (*d*)

Table I *Results of coagulation assays and the daily doses of heparin given*

Coagulation assays	March 4	March 5	March 7	June 11	Normal values
Thrombocytes (counts/μl)	208 000	200 000		288 000	150 000–400 000
Normotest (%)	14	105	110	10	70–130
Thrombotest (%)	9	72	105	22	70–130
Cephalin time (sec)	63	54	75		54–62
Cephotest (sec)	44 5	31 5	57 5	30	28–31
Quick time (sec)	28	16	18	26	14–16
Thrombin clotting times (sec)	18	20	180	19 5	18–20
Factor VIII activity (%)	100				
Fibrinogen (mg/dl)	400			330	150–400
Ethanol gelation test*	+	+	–	–	–(negative)
FDP (Thrombo-Wellcotest)	+1/20	+1/20	+1/5	–1/5	–1/5
Heparin (IU/24 h)		40 000	60 000		

Reference 2

min K (Konakion®) 10 mg i v to counteract the effect of warfarin Because of the possibility of thrombotic involvement of the left renal vein laparotomy was performed The iliac veins and the inferior vena cava were packed with an old organized thrombus reaching up to the renal veins The top of the thrombus was floating in the blood stream and reached beyond the renal veins without occluding them A marked phlebitis was present both in the vena cava and in the iliac veins The right spermatic vein was occluded by a fresh thrombus without phlebitic changes in the vessel wall The left spermatic vein was open

The vena cava was cut 5 mm below the renal veins The thrombus in the upper part of the vena cava was removed and the proximal end of vena cava closed with a continuous suture flush with the renal veins The occluding thrombus in the lower part of the vena cava and the iliac vessels was organized and could not be removed Before closing the abdominal wall a balloon catheter was placed in the bladder through a cystostomy

Heparin was given for eight days Thereafter oral anticoagulation with warfarin was started and heparin was discontinued when a therapeutic value of TT had been obtained

The wound healed primarily and the edema of the lower extremities and the genitalia disappeared within three weeks In spite of a restored circulation to the penis several superficial ulcerations persisted on glans penis and a deep gangrenous ulceration about 3 cm in diameter on the ventral side of the shaft of penis (Fig 1*b*) The necrotic tissue was excised and a skin transplantation performed When the cystostomy catheter was removed 4½ weeks postoperatively the patient passed urine normally He was discharged one week later Anticoagulation with warfarin was continued and elastic stockings during the day were recommended

At a routine check three months later there was no significant edema but skin pigmentation and moderately dilated subcutaneous veins had developed in both legs (Fig 1*d*) He passed urine normally and his sexual function was normal Coagulation assays demonstrated no evidence of DIC (Table I)

DISCUSSION

Profound edema of the external genitalia may result from thrombosis of the inferior vena cava (1 6) but gangrene of the penis does not seem to have been reported earlier It is possible that the penis gangrene in our patient was related to the acute thrombotic obstruction of the right spermatic vein in addition to obstruction of the inferior caval vein The rapid development of the gangrene in conjunction with the finding of a fresh non adherent thrombus in the right spermatic vein favours this assumption

In this patient thrombosis of the inferior caval vein was accompanied by widespread superficial thrombophlebitis in both arms and the coagulation assays were indicative of DIC Because no underlying disease was found it is suggested that DIC might have resulted from activation of coagulation of the circulating blood in the vicinity of the thrombus As shown our patient had an increased heparin tolerance Increased heparin tolerance has been reported in patients with pulmonary thromboemboli (4) but may be seen in any patient with major venous thrombosis

The most common symptoms following thrombotic occlusion of the lower segment of the inferior caval vein are related to venous insufficiency of the lower extremities resulting in chronic edema varicosities chronic dermatitis and ulcerations (6 7 9 11) Our patient had only insignificant edema of the legs but he had developed dermatitis and skin pigmentations (Fig 1*d*) In spite of impending gangrene of the penis he has regained normal sexual functions

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Post-Transfusion Purpura Treated with Plasma Exchange by Haemonetics Cell Separator

A Case Report

B Laursen N Morling J Rosenkvist H Sørensen and S Thyme

From Department of Medicine A Division of Haematology and the Blood Bank Tissue Typing Laboratory Rigshospitalet University Hospital Copenhagen Denmark

STRACT A case of post transfusion purpura in a year old multiparous female with a platelet al ntibody (anti Zw) in her serum is reported The tient was successfully treated with plasma ex nge by means of a Haemonetics 30 cell separator corticosteroids Compared with other therapeu measures, plasma exchange seems to shorten the ation of thrombocytopenia Major surgery was sible in our patient within ten days of development e syndrome

t transfusion purpura (PTP) is a rare syndrome by Shulman et al (15) It is characterized thrombocytopenic purpura developing approx tely one week after blood transfusion and the sence in the patient s serum of an alloantibody st the platelet antigen Zw[a] (Pl[A1]) present in it 98% of donors The extraordinary fact is it this antibody causes in vivo destruction not ly of donor platelets but also of the Zw[a] negative elets of the recipient

The haemorrhagic disorder was fatal in one (6) of 19 previously published cases two have been d with exchange transfusion of whole blood 15) and one with plasmapheresis (1) In the maining cases the disease appears to have been lf limited with complete recovery within 50 days ll patients but one were treated with cortico roids and all but one were females

In the present study we describe the course of TP in a patient in whose serum a complement xing Zw[a] antibody was demonstrated The patient as successfully treated with plasma exchange erformed with a Haemonetics 30 cell separator and with corticosteroids It was thus possible to perform major surgery within ten days of the development of thrombocytopenia

METHODS

Routine laboratory tests were performed by standard methods in the Department of Clinical Chemistry

Platelet complement fixation test was made according to Colombani et al (4)

HLA typing and assays of *lymphocytotoxic antibodies* were made according to Kissmeyer Nielsen and Kjerbye (8)

Plasma exchange was done by means of a Haemonetics 30 cell separator with a 375 ml bowl All blood was anticoagulated with ACD (formula A one part ACD+7 parts blood) and after separation the patient s erythrocytes were reinfused with equivalent volumes of donor plasma

CASE REPORT

A 61 year old woman was transferred from another hospital due to severe thrombocytopenia and haemorrhagic diathesis There was no previous history of haemorrhages or thromboembolism and the patient had never received blood transfusions She had completed four uncomplicated pregnancies with three normal deliveries and one stillbirth One abortion

Present illness Fifteen days prior to transfer the patient was admitted to the local hospital for evaluation and treatment She had a 3–4 months history of intermittent abdominal pain constipation anorexia and weight loss X ray of the colon revealed a stenosing process in the ileo-coecal region Clinically and by laboratory tests there were no signs of metastases

Correspondence to B Laursen M D Dep Medicine A Rigshospitalet DK 2100 C Denmark

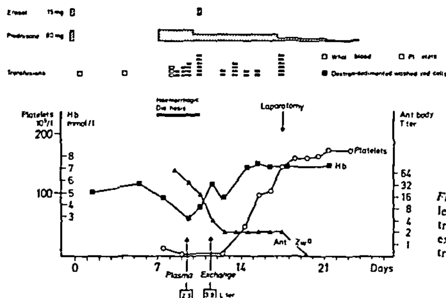


Fig 1 Hb and platelet levels antibody titre no of transfusions days of plasma exchange and prednisone treatment

As part of the preoperative treatment the patient was given her first transfusion one unit of whole blood This was followed by chills and a rise in temperature to >39°C Four days later the second transfusion of whole blood was started but had to be stopped due to chills and fever

Seven days after the initial transfusion the patient suddenly developed innumerable petechiae on the body and extremities oozing from gingivae and melaena A platelet count of 8×10^9/l was found prothrombin proconvertin of 0 80 Treatment with prednisone 80 mg/day was initiated Twenty four hours later the patient was transferred to this hospital

n admission the patient appeared chronically ill with undice but anaemic with countless petechiae on the and extremities oozing from 3+ and 4+ At this e there was neither melaena nor haematuria The abdomen was distended and tender without peritoneal reaction A mass was palpable in the right iliac fossa the liver was not enlarged the spleen 2-3 cm below the left costal margin Hb was 3 3 mmol/l (normal range 7 0-9 4) platelet count 1×10^9/l (normal range 160-400) all tests for consumption coagulopathy were negative Treatment with prednisone transfusion and plasma exchange is shown in Fig 1 and described in detail below In addition the patient was given penicillin 5 mill U×4 daily gentamycin 60 mg×4 daily

The day after transfer the patient developed haematuria and manifest ileus Since surgery at this time was impossible she was given Erasol® (H_1N) in an attempt to decrease the intestinal obstruction Post or propter the ileus subsided From one day after the plasma exchange haematuria ceased and the petechiae gradually disappeared

Nine days after transfer the patient was operated on with an ileostomy and appendectomy The surgeon found an abscess around the vermiform appendix and coecum and an obstructing tumour of the proximal part of the ascending colon with enlargement of the mesenteric glands There was no abnormal haemorrhage per and postoperatively The postoperative course was unremarkable and the patient was discharged to her home on the 15th postoperative day

Microscopy Appendix in parts infiltrated with adenocarcinoma both in the mucosa submucosa and mesentery of the vermiform appendix In parts the tumour was mucine producing *Bone marrow* cellular with increased normoblastic erythropoieses left shift granulopoieses and few megakaryocytes No tumour cells were seen

Laboratory studies

Hb and platelet values are shown in Fig 1 On admission P-P 0 73 (normal range 0 85-1 15) fibrinmonomer 0 partial thromboplastin time 40 9 (control 63 1) fibrinogen thrombin time euglobulin clot lysis time fibrinogen related antigen within normal limits leucocyte count 1 (normal range 3 0-8 5) differential count normal Alkaline phosphatase and blood urea normal IgG IgM IgA within normal limits

Serological investigations

The patient s tissue type was HLA A 1,2 B8 w22 and her serum contained a weak (titre 1) lymphocytotoxic anti HLA B7 antibody No irregular blood group antibodies were detected and Coombs direct antiglobulin test was negative

On admission the patient s serum was tested for complement fixing platelet antibodies against a panel of selected individuals Sixteen were Zw[a]-positive and gave strongly positive reactions (titre 64) A negative reaction was found with platelets from a female who had previously been treated for PTP (20) The Zw[a] specificity was further confirmed by negative reactions with platelets from another Zw[a] negative female (provided by F Kissmeyer Nielsen Tissue Typing Laboratory Kommunehospitalet Århus) previously identified as Zw[a]-negative with the anti Pl[A1] serum from Shulman (19) and with platelets from the patient herself after recovery

le I *Summary of reported cases of post transfusion purpura*

	Sex	Age (y)	No of previous preg nancies/ trans fusions	No of initial trans fusions	Day of onset of PTP after trans fusion	Duration (days) Pur pura	Thrombo- cyto- penia	Purpura/ thrombocyto- penia after exchange
Loghem et al (9)	♀	51	3/0	1	7	8	21	
ker et al (22)	♀	56	3/0	1	5	26	26	
lman et al (15)	♀	40	3/0	3	7	3	6	0/3
lman et al (15)	♀	43	3/0	2	6	20	30	
lman (14)	♀	41	?	6	3–7	3	24	
nson & Mollison (10)	♀	55	4/0	2	6	35	40	
(11)	♀	78	2/+	2+2	4–6	10	11	
gaard et al (19)	♀	39	1/1	3	8	8	12	
gaard (18)	♀	64	4/1	2	7	12	50	
arry et al (5)	♀	47	2/?	1+1+1	3–8	9	14	
olls et al (12)	♀	68	0/0	3+4	3–6	14	21	
& Aster (3)	♀	77	8/0	2	8	6	10	1/4
man & Shulman (6)	♀	49	2/0	3	7	1[a]		
amson et al (1)	♀	45	+/?	1+2	5–7		12	–/4–7
ler et al (21)	♀	34	0/5	1+1	5–11		35	
ler et al (21)	♂	53	–/2	6	8		11	
ler et al (21)	♀	51	+/+	4+1	9		9	
ard et al (7)	♀	70	+/0	2	7		18	
nsen & Thyme (20)	♀	53	2/0	4	6	7	32	
nt paper	♀	61	5/0	1+1	4–7	4	10	1/7

e patient died after 24 hours

tion the patient s serum was tested with platelets 203 random donors Of these 198 (97 5%) gave reactions corresponding to the previously found ency of the platelet specific Zw[a] antigen (9)

from the patient s two living children were to be Zw positive and reacted with the patient s

lly by means of a complement consumption assay circulating immune complexes were demonstrated in s serum prior to plasma exchange (by S E ag Institute for Medical Microbiology Odense)

sion and plasma exchange

e first two days after transfer the patient was treated *transfusion* of platelet rich plasma from random and with leucocyte poor dextran sedimented red cell suspensions (Fig 1) Platelet transfusions discontinued after the serological identification of body in the patient s serum

order to decrease the antibody titre in the patient s and to remove any immune complexes that might esent *plasma exchange* was undertaken on *day nine* the initial blood transfusion Plasma from the patient substituted with fresh donor plasma Unintentionally irst units used were platelet rich and the patient de ped a febrile reaction Consequently it was seen to all subsequent plasma units were centrifuged at 6456 ottom of the bowl) for 5 min to provide as nearly as ible cell free samples All these plasma units except were given without reaction The reacting unit was found to contain a strong specific HLA A1 antibody day 11 after the initial blood transfusion a second plasma exchange was completed without any side effects

During the first plasma exchange 2265 ml patient plasma were substituted with 2090 ml donor plasma during the second plasma exchange the volumes were 3845 ml and 3950 ml respectively Donor plasma was AB0- and Rhesus-compatible Neuromuscular hyperexcitability due to citrate induced hypocalcaemia developed on one occasion and was treated with 0 5 g calcium laevulat (Sandoz)

RESULTS

After the first plasma exchange the titre of the anti Zw[a] decreased from an initial level of 64 to 16 and after the second plasma exchange to a level of 2 The antibody was still detectable for 6 more days (Fig 1)

One to two days after the second plasma exchange an increase in platelet count occurred and normal values were reached within 6 days (Fig 1) The thrombocyte level remained normal during surgery and the rest of the observation period

DISCUSSION

The original work by Shulman et al (15) which demonstrates a decreased survival of Zw[a] platelets in the immunized patient and the

Table II *Statistical summary of the effects of blood/plasma exchange versus no exchange*

	Purpura			Thrombocytopenia		
	Duration (days)			Duration (days)		
	Median	Range	No of pats	Median	Range	No of pats
Blood or plasma exchange	4	3–6	3	10	6–12	4
No exchange	10	3–35	11	21	9–50	15
Mann-Whitney test		$0.02<p<0.05$			$0.01<p<0.02$	

tion by later authors of the simultaneous occurrence of PTP and specific platelet antibodies leave little doubt that the antibody is the most important aethiological factor in this disease All patients but one have been found to be Zw^a negative and to have developed the specific Zw^a antibody Recently however Zeigler et al (21) reported a case of PTP in a Zw^a positive nulliparous woman who clinically and laboratorywise fulfilled the criteria for the diagnosis of PTP (development of thrombocytopenic purpura approximately one week after transfusion the presence in her serum of a complement fixing antibody reacting with donor but not with her own platelets following recovery and the decrease in ^{51}Cr release from normal platelets after recovery) Apart from this anti Zw^a been found only in patients who have received transfusions and in women who have been pregnant given birth to children suffering from isoimmune neonatal purpura

In our laboratory several hundreds of sera from patients who have had transfusion reactions have been investigated by use of the complement fixation test The only two individuals in whom Zw^a antibodies have been demonstrated are the present case and one previously published patient with PTP (20) However it is possible that platelet specific antibodies may not be detectable by the complement fixation test although demonstrable by other techniques such as the ^{51}Cr release technique (2 21) and the platelet indirect radioactive Coombs test (16 17)

The mechanism of platelet lysis in PTP has not yet been elucidated As proposed by Shulman et al (15) circulating immune complexes may form between antigen from the transfused Zw^a positive platelets and anti Zw^a in the recipient s serum These complexes may be adsorbed non specifically onto the patient s Zw^a negative platelets thus causing destruction of these either by activated complement or by phagocytosis in the reticuloendothelial system Another possible explanation is that the Zw^a antibodies cross react with some antigen of the Zw^a negative thrombocytes of the patient (10)

In the patient presented in this study carcinomatous infiltration of the bone marrow and disseminated intravascular coagulation had to be considered as possible causes of the thrombocytopenic purpura However since no positive evidence of these causes was obtained and the patient fulfilled the criteria for the diagnosis of PTP we decided to treat her with plasma exchange favouring the opinion that circulating antigen–antibody complexes are of pathogenetic significance in this syndrome

In accord with the findings in previous cases treated with exchange transfusions (1 3 15) the antibody titre of our patient did not show a dramatic decrease as a result of the plasma exchange and anti Zw^a was still detectable at the time when the thrombocyte count increased Circulating immune complexes were found in a blood sample taken from this patient when plasma exchange was initiated Unfortunately we were unable to quantitate immune complexes during the course of plasma exchange

In spite of the fact that in this patient the haemorrhagic diathesis disappeared and the platelet count rose concurrently with the plasma exchange it is not possible at present to evaluate conclusively the significance of this treatment in patients with PTP However as to the duration of thrombocytopenic purpura in patients with PTP a review of the data published until now indicates a shorter course in patients who have been treated with exchange of whole blood or plasma (Tables

1 II) We are well aware of methodological alls that may invalidate this opinion but suggest a repeated plasma exchange should be tried in ients suffering from PTP as it may prove to be effective treatment and to shorten the duration a thrombocytopenia with the risks of severe morrhages At the same time further investiga ns should be made as to the quantity and compo- nts of circulating immune complexes

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LETTERS TO THE EDITOR

CARDIAC ARRHYTHMIA DUE TO CHLORAL HYDRATE

Dear Sir

I congratulate Dr Gustafson and his associates for the excellent paper describing three cases of cardiac arrhythmia induced by chloral hydrate (3) It is rather fortunate that this particular type of cardiac complication due to chloral hydrate overdosage is infrequent In addition to the four reports of cardiac arrhythmia caused by chloral hydrate that Dr Gustafson et al referred to in their paper (1 2 5 6) I wish to bring the following to the attention of the authors under reference and the readers of your journal

After Nordenberg et al (6) described the first case of cardiac arrhythmia in a child two more pediatric cases of cardiac complications viz sinus arrhythmia were reported in a 13 and 18-month-old child following an intake of 750 and 500 mg of chloral hydrate respectively (7) Subsequent to this Vazin et al (8) reported the first and only case of arrhythmia in a pregnant woman who ingested 17 5 g of chloral hydrate in a suicidal attempt The cardiac irregularities in this case consisted of ventricular fibrillation periods of asystole cardiac arrest and sinus tachycardia with frequent multiple multifocal premature ventricular contractions The latter had persisted despite treatment with i v lidocaine The patient was treated with hemodialysis

As a postscript I must update the information by referring to the two cases of arrhythmia due to chloral hydrate which have been published after the article by Dr Gustafson et al in the 15 Oct 1977 issue of Br Med J (4)

I hope that the above information will be of interest use to your readers at large

Jota S Ghosh MS 811 Clifford Avenue Ardmore 19003 USA

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Sir

I have read with great interest the article entitled "Sudden death in rheumatoid arthritis with atlanto-axial dislocation" by I Mikulowski et al (Acta Med Scand 198 445–451 1975)

Sudden unexpected death in young adults is one of the perplexing and bewildering problems in medicine This article throws light from one of the regions which is often neglected in the post mortem examination of sudden unexpected death cases namely the cervical spine As pointed out in the article one of the important causes of sudden death in rheumatoid arthritis is atlanto-axial dislocation

Another condition in which cervical spine pathology may be the cause of sudden unexpected death is carcinomatous metastasis to the cervical vertebrae Lately I have performed an autopsy on the cadaver of a middle aged woman who suffered from carcinoma mammae with known metastatic involvement of the liver spinal column The woman was medically treated and s was in a relatively good condition She died suddenly immediately after stepping down from a small rock during a walk in a park Post mortem examination disclose pathological fracture and dislocation of the cervical spi column with compression of the cord The cervical ver tebrae were severly involved by the carcinomatous cess

It should be stressed that thorough examination of th cervical spine may be of the utmost importance in som cases of sudden unexpected death and failure to do thi may leave the cause of death undisclosed

Yours

Karl Otto Schwarz M D
Leopold Greenberg Institute of Forensic Medicine
Jaffa Israel